

FIGURE 3. Effect of adaptation to short stresses and of prolonged EPS on induction of lipid peroxidation in liver with Fe/ascorbate. The abscissa is time after induction in minutes. The ordinate is malonic dialdehyde content in nanomoles per milligram protein. (1) Control, (2) EPS, (3) adaptation, (4) adaptation plus EPS.

acted the stress induction of peroxidation, most probably owing to enhanced activity of antioxidant systems.

Further, it was confirmed that hepatic stress damage involves inactivation of cholesterol 7- α -hydroxylase, and this is preventable with prior adaptation.

On the whole, adaptation to short stresses can prevent not only myocardial stress damage, but also hepatic stress damage and thereby atherogenic dyslipidemia, which is a weighty pathogenetic factor of coronary sclerosis and ischemic heart disease.

The importance of these data is determined by the fact that the stress reaction participates virtually in all pathogenetic links of ischemic disease (see Figure 8 in Chapter 2). Hence, adaptation that protects the organism from stress damage must also protect the heart from ischemic damage.

TABLE 7

Effect of Emotional Pain Stress (EPS), Adaptation to Stress, and Antioxidant on Hepatic Content of Malonic Dialdehyde (MDA), Hepatic Superoxide Dismutase (SOD) Activity, and Fructose 1,6-Diphosphate Aldolase (FDA) Activity in Blood

Series	MDA (nmol/g prot.)	SOD (U/g prot.)	FDA (U/ml)
Control N = 9	300 ± 30	64.6 ± 7.3	124 ± 80
EPS, 2 h after (n = 8)	640 ± 50 <i>p</i> ₁₋₂ <0.001	30.8 ± 2.0 <i>p</i> ₁₋₂ <0.001	316 ± 20 <i>p</i> ₁₋₂ <0.001
EPS, 24 h after (n = 8)	370 ± 20 <i>p</i> ₁₋₃ >0.05	50.3 ± 3.9 <i>p</i> ₁₋₃ >0.05	282 ± 20 <i>p</i> ₁₋₃ <0.001
Adaptation (n = 10)	340 ± 10 <i>p</i> ₁₋₄ <0.05	93.8 ± 4.3 <i>p</i> ₁₋₄ <0.01	140 ± 10 <i>p</i> ₁₋₄ <0.05
Adaptation + EPS 2 h after (n = 8)	370 ± 40 <i>p</i> ₂₋₅ <0.001	85.5 ± 5.6 <i>p</i> ₂₋₅ <0.001	176 ± 22 <i>p</i> ₂₋₅ <0.001
Adaptation + EPS 24 h after (n = 7)	290 ± 10 <i>p</i> ₄₋₆ >0.05	80.6 ± 6.4 <i>p</i> ₄₋₆ >0.05	—
Ionol (n = 9)	330 ± 20 <i>p</i> ₁₋₇ <0.01	96.2 ± 4.1 <i>p</i> ₁₋₇ <0.01	100 ± 20
Ionol + EPS, 2 hr after (n = 8)	350 ± 20 <i>p</i> ₂₋₈ <0.001	78.1 ± 3.8 <i>p</i> ₂₋₈ <0.001	120 ± 80 <i>p</i> ₂₋₈ <0.001
Ionol + EPS, 24 h after (n = 8)	290 ± 10 <i>p</i> ₁₋₉ >0.05	68.1 ± 2.6 <i>p</i> ₁₋₉ >0.05	—

IV. ADAPTATION TO STRESS PREVENTS AND ABOLISHES DISTURBANCES TO CARDIAC CONTRACTILE FUNCTION AND ELECTRIC STABILITY IN ACUTE ISCHEMIA, REPERFUSION, MYOCARDIAL INFARCTION, AND POSTINFARCTION CARDIOSCLEROSIS

With a view to using adaptation as a cardioprotector against stress, it was deemed expedient to ascertain first how adaptation affects the cardiac contractile function and tolerance to load and hypoxia in the whole organism.

Together with Belkina we studied the rat heart contractile function *in situ* by electromanometric recording of the left ventricular pressure at physiological rest and under load imposed with aortic clamping.

The main results presented in Figure 4 bring out two issues. First, adaptation to short immobilization stresses did not appreciably alter the left ventricular contractile function at relative physiological rest; there was only a tendency to decreasing developed pressure, contraction rate, and relaxation velocity. Second and most important, the adaptation substantially enhanced the heart resistance to maximal load: at the 5th second of aortic clamping all contractile parameters were practically similar in control and adapted animals, and at the 25th second the depression of the contractile function was pronounced in the controls, but insignificant in the adapted rats. As a result, the adapted animals exceeded the controls in developed pressure by 43%, in IFS by 77%, in contraction velocity by 80%, and in relaxation velocity by 43%. It is noteworthy that the higher contractile function in adapted animals was retained with the diastolic pressure decreased almost by 40%, and therefore can hardly be attributed to a more effective operation of such self-regulatory mechanisms as the Starling mechanism.

It should be borne in mind that adaptation to repeated stress consistently entails steady-state activation of catecholamine synthesis in the brain and adrenal glands, i.e., in the long run, increased power of the sympathoadrenal system. It can therefore be thought that one of the factors providing for the maintenance of cardiac function under maximal load in

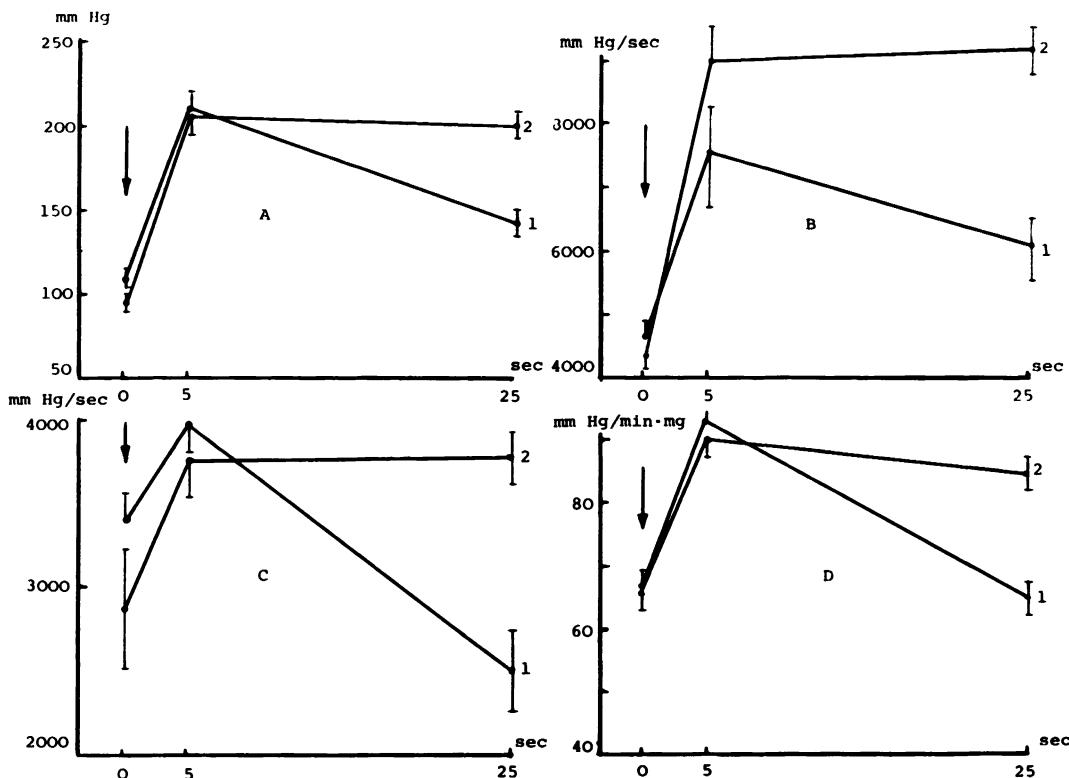


FIGURE 4. Effect of adaptation to short stress exposures on the rat left ventricular contractile function upon aortic clamping. The abscissa is time after clamping in seconds. The ordinates are (A) developed pressure, mmHg; (B) maximal pressure buildup rate, mmHg/s; (C) maximal pressure drop rate, mmHg/s; (D) IFS, mmHg/min·mg. (1) Control, (2) adaptation.

adapted animals is the more stable positive inotropic influence of the adrenergic system on the heart. There is, however, no doubt that a no less important part in the protective adaptational effect can be played by increased myocardial resistance to hypoxia; so at the next step of our study we considered the effect of adaptation to stress on cardiac resistance to hypoxic hypoxia in rats created *in situ* by switching off the respiration apparatus.

In this work carried out together with Golubeva and Kopylov, we used the following criteria: creatine phosphate (CP) content and creatine phosphokinase (CPK) activity, adenine nucleotide content, glycogen content, and phosphorylase activity. In the course of the acute experiment, after stabilization of the cardiac contractile function the apparatus for controlled respiration was switched off for 4 min to create hypoxic hypoxia; then it was switched back on for reoxygenation. For biochemical studies the hearts were instantly frozen in liquid nitrogen with the Wollenberger forceps (1) before hypoxia, in stable function; (2) at the end of the fourth minute of hypoxia, in depressed contractile function; and (3) at the end of the fifth minute of reoxygenation, when the function was to some extent restored.

The data in Table 8 show that, as in earlier published works, in control nonadapted animals hypoxia decreased the ATP content by more than 30%, CP by an order of magnitude and glycogen almost threefold, whereas lactate more than doubled. Concurrently, CPK reliably declined by 16% and there was a tendency to a decrease in total phosphorylase. In reoxygenation the CP and ATP levels were restituted to about 80% of initial values, and glycogen and lactate did not reverse at all; CPK remained at the hypoxic level, while phosphorylase continued to decrease and finally fell by more than 20%. Thus, the most

TABLE 8
Effect of Adaptation to Short Stress Exposures on The Indices of Cardiac Energy Metabolism in Acute Hypoxia and Reoxygenation

Indices	Control			Hypoxia	Reoxygenation
	Initial	Hypoxia	Reoxygenation		
CP (μmol/100 g tissue)	614 ± 42	60 ± 15	479 ± 49	592 ± 30	56 ± 12
ATP (μmol/100 g tissue)	412 ± 28	284 ± 17	328 ± 21	411 ± 30	276 ± 29
Glycogen (mg/100 g tissue)	408 ± 29	142 ± 16	153 ± 17	502 ± 76	166 ± 38
Lactate (μmol/100 g tissue)	1.55 ± 0.11	3.84 ± 0.29	3.40 ± 0.41	1.61 ± 0.40	3.45 ± 0.67
CPK (mmol/min·100 g tissue)	54.4 ± 1.8	46.0 ± 1.0	47.7 ± 2.1	52.2 ± 2.5	52.9 ± 2.9*
Total phosphorylase (A + B)	4002 ± 134	3528 ± 231	3192 ± 272	4668 ± 347	4401 ± 401*
Phosphorylase A/(A + B) ratio	0.18 ± 0.02	0.31 ± 0.03	0.17 ± 0.02	0.18 ± 0.02	0.37 ± 0.04
					0.18 ± 0.04

* Difference from respective controls statistically significant; n = 9 ÷ 16 in different series.

interesting feature of the results in control animals is the moderate but persistent decline in vital enzymes of energy metabolism — CPK and phosphorylase — which is not abolished in reoxygenation.

Animals adapted to stress displayed the same complex of disturbances of energy metabolism typical of acute hypoxia (decreasing ATP, CP, and glycogen, increasing lactate), but no hypoxic decline in CPK and phosphorylase. Still more marked differences revealed themselves in reoxygenation: the adapted animals maintained both enzymic activities and achieved complete recovery of ATP and superrecovery of CP.

Physiological experiments showed that hypoxic hypoxia depresses the cardiac contractile function in both control and adapted animals. Yet it is evident in Figure 5A that by such an integral index as IFS the adapted animals at the end of the fourth minute of hypoxia exceed the controls almost by half ($p < 0.05$). Accordingly, they restored the contractile function in reoxygenation much sooner than did the controls and, as in the case of the CP content, with superrecovery by the end of reoxygenation. A similar pattern was observed for another important index, contraction velocity (Figure 5B).

Thus, adaptation to stress enhances the stability of vital energy-metabolic enzymes CPK and phosphorylase in hypoxia and reoxygenation, and accelerates the restitution of contractile function in reoxygenation after hypoxic hypoxia. The results is enhanced resistance of the cardiac muscle to loading and hypoxia.

On this basis we studied the effect of prior adaptation to short stresses on the impairment of contractile function usual in experimental myocardial infarction. It was found that upon myocardial infarction produced according to Selye et al.,³⁸ such important indices of left ventricular function as developed pressure, IFS, pressure buildup, and drop rates decreased at rest by 25 to 30%. In adapted animals the depression of these parameters did not reach statistical significance. This effect was maintained under maximal load on the heart created by complete clamping of the aorta for 30 s. At maximal load on the left ventricle with an infarct, the effect of prior adaptation proved more pronounced than with the normal heart. Indeed, at the 25th second of aortic clamping the contractile indices in adapted infarction animals exceeded those in nonadapted infarction animals by the following factors: left ventricular developed pressure, 2.8 ($p < 0.001$); IFS, 3.8 ($p < 0.01$); maximal pressure buildup and drop rates, 3.7 and 3.25, respectively ($p < 0.001$) (Figure 6).

There is thus no doubt that prior adaptation to short stress episodes markedly restricts the impairment of left ventricular contractile function in experimental infarction and is an efficient protection against ischemic damage. This protective effect can be due to attenuation of the necrotic zone as well as to prevention of injury to nonischemic heart regions usual in infarction. We studied both possibilities.

To assess the influence of adaptation on nonischemic regions, we measured the function of right auricles isolated from adapted and nonadapted animals with left ventricular infarction. The adaptation as such did not appreciably affect the right atrial contractile function, but substantially attenuated its depression caused by left ventricular infarction (described in detail in Chapter 2, Section IV). In fact, in nonadapted animals the ventricular infarction decreased the maximal values of atrial developed force and IFS 2 to 2.5-fold, whereas in adapted ones the decrease was less than one third. Thus, prior adaptation to short stresses certainly lessened the functional impairment of nonischemic myocardial regions in experimental infarction.

Further experiments presented in Chapter 6 have shown that adaptation to repeated stress is accompanied by another cardioprotective effect, namely attenuation of the necrotic zone in experimental myocardial infarction.

Hence it can be stated that *the protective effect of prior adaptation to stress in experimental infarction comprises two components: restriction of the stress damage to nonischemic heart regions, and cytoprotective action attenuating the necrotic volume of the myocardium.*

It must, however, be emphasized that the adaptational protection of the heart against

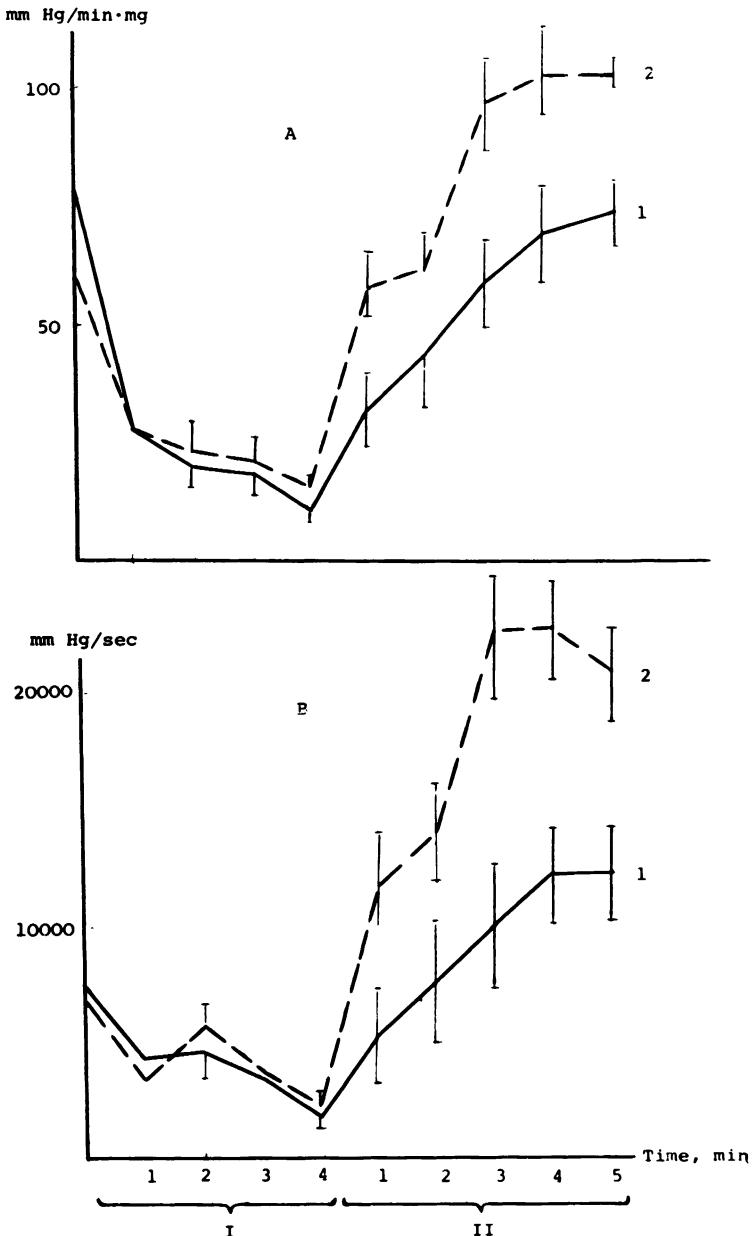


FIGURE 5. Effect of prior adaptation to stress on the dynamics of cardiac contractile function in anoxia and reoxygenation. The abscissa is time in minutes of (I) anoxia and (II) reoxygenation. The ordinates are (A) IFS, mmHg/min·mg; (B) maximal pressure buildup rate, mmHg/s. (1) Control, (2) adaptation.

ischemic damage cannot be reduced to mere restriction of necrosis. Such adaptation has been shown above to protect first and foremost against stress which always accompanies acute ischemia, reoxygenation, or myocardial infarction and has a profound arrhythmogenic action. In accord with this, our further studies demonstrate that adaptation to noninjurious short stress exposures furnishes a high degree of cardiac protection from arrhythmias common in acute ischemia, reperfusion, myocardial infarction, and postinfarction cardiosclerosis.⁵

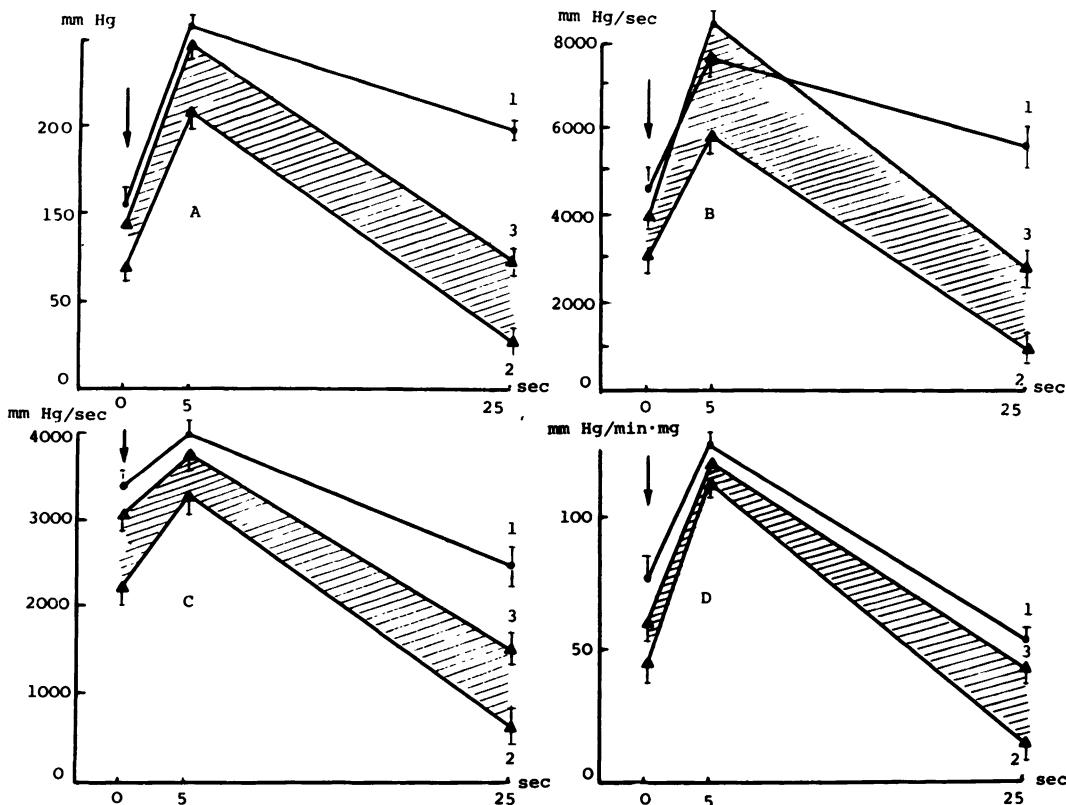


FIGURE 6. Effect of adaptation to stress on left ventricular contractile function of rat hearts with acute myocardial infarction upon aortic clamping. The abscissa is time after clamping in seconds. The ordinates are (A) developed pressure, mmHg; (B) maximal pressure buildup rate, mmHg/s; (C) maximal pressure drop rate, mmHg/s; (D) IFS, mmHg/min·mg. (1) Control, (2) infarction, (3) adaptation, (4) adaptation plus infarction. The shaded zone denotes the protective effect of adaptation.

Most of the data on impairment of the cardiac electric stability in response to coronary artery occlusion have been obtained under conditions quite far from natural (anesthesia, opened chest). Obviously, anesthesia would suppress the stress effect of ischemia, whereas thoracotomy would complicate it, which could make equivocal the evaluation of the effects of antistress factors on the cardiac electric stability. Therefore, we carried out our studies on the effect of acute ischemia on cardiac rhythm disorders and their prevention with closed-chest conscious animals using the technique of Lepran et al.,⁴⁸ which included two steps. First, under anesthesia a ligature was drawn under the left coronary artery and led out through a special cannula to just beneath the skin, but not tightened. Second, 3 to 5 d after these animals were fixed in the supine position while conscious, a skin incision was made and the ligature tightened on the coronary artery for 5 or 20 min. The incidence of various arrhythmias — fibrillation, ventricular tachycardia, and extrasystoles — and their overall duration were determined in control animals and those protected with adaptation to short stresses or with ionol.

In the first 5 min of ischemia, most of the controls (14 of 20) developed severe arrhythmias. In adapted animals, ventricular fibrillation was not observed at all, ventricular tachycardia only in 2, and extrasystole in 4 out of 20 rats. The difference was further deepened by the duration of arrhythmias in adapted animals being significantly less than in nonadapted ones. Thus the overall duration of arrhythmias was 541 s in the control, but only 36 s (i.e., 15 times less) in adapted rats.

One can see that *adaptation to short stress episodes has a pronounced prophylactic effect against stress-induced and ischemic impairment of the electric stability of the heart: it prevents the decline in the fibrillation threshold in stress, the rise in ectopic activity, and fall in the fibrillation threshold in myocardial infarction, and the fibrillation itself in acute ischemia under consciousness.*

Then we used adaptation to stress to prevent the impairment of the cardiac electric stability in myocardial infarction. Experimental infarction was produced according to Selye et al.,³⁸ as described above in adapted and nonadapted animals, to compare such alterations typical of infarction as lowering of the ventricular fibrillation threshold and appearance of ectopic extrasystole in vagal inhibition of the cardiac pacemaker.

Table 9 shows that 2 d after infarction the nonadapted animals had the same heart rate, but decreased arterial pressure and a more than threefold lower fibrillation threshold, which agrees with the experimental and clinical data of other researchers.^{51,52} Adaptation to stress did not, *per se*, change the fibrillation threshold, but substantially prevented its postinfarction decrease. Since a lower fibrillation threshold means a higher proneness of the heart to arrhythmias, these data indicate that adaptation substantially reduces the probability of cardiac fibrillation in the late period of acute myocardial infarction.

As can be seen in Figure 7, upon infarction there is also a decline in the resistance of the sinus node automatism to vagal inhibition, which is especially clear with vagal stimulation currents of two to three thresholds. Adaptation as such attenuates the negatively chronotropic vagal effect (curves 3 vs. 1), and in infarction its action is still more pronounced (curves 4 vs. 2).

It is essential that upon vagal stimulation, 70% of nonadapted infarction animals developed extrasystoles as compared to only 18% in the controls. This appears to be due to a deeper vagal inhibition of the sinus node automatism in infarction as well as to appearance of ectopic excitation foci in the necrotic border zone which reveal themselves upon suppression of the normal pacemaker. Such phenomena have been observed by researchers^{53,54} and vagal stimulation is used as a means of detecting the latent ectopic excitation foci.^{53,55,56} Our experiments show that prior adaptation to stress lowers the cardiac ectopic activity in myocardial infarction; just 18% of adapted infarction animals developed extrasystoles. Moreover, the total number of extrasystoles in adapted animals with infarction was even less than in controls. It is known that ectopic activation not only leads to extrasystoles, but is also a necessary link of spontaneous fibrillation.⁵³ Hence, the adaptational suppression of ectopic automatism means a lower probability of cardiac arrhythmias and fibrillation in the postinfarction period.

Thus, *adaptation to short stresses prevents the postinfarction decline in the ventricular fibrillation threshold and appearance of ectopic excitation foci, increasing the sinus node resistance to vagal inhibition.*

This promising result naturally gave rise to a question whether or not such adaptation can abolish the already existing disturbances of the electric stability when the detrimental factors proper — stress and ischemia — have long ceased to act and there is only the result of infarction, namely vast postinfarction cardiosclerosis. Indeed, as long ago as 1959 Sumarokov⁵⁷ showed that doses of epinephrine that in most normal dogs cause only transient bradycardia, in animals with postinfarction cardiosclerosis evoke paroxysmal ventricular tachycardia and ventricular extrasystole. To solve this question, adaptation to short immobilization stresses was started 15 d after experimental myocardial infarction, when the postinfarct scar had already mostly formed in the left ventricle. Adaptation was carried on for 2 weeks and then (1 month after infarction) adapted and nonadapted animals were tested for the fibrillation threshold and ectopic cardiac activity in acute experiments. By that time the postinfarct scar in the left ventricular myocardium was clearly defined visually and its weight was about the same in both series, varying from 151 to 165 mg.

TABLE 9
**Prevention of the Postinfarction Decline in Ventricular Fibrillation Threshold
with Prior Adaptation to Short Stress Exposures**

Series	Heart rate (min ⁻¹)	Arterial pressure		Fibrillation thresh- old (mA)
		Systolic	Diastolic	
Control (n = 11)	404 ± 13	1427 ± 7	87 ± 12	7.0 ± 0.4
Infarction (n = 10)	394 ± 17	90 ± 7	66 ± 11	2.1 ± 0.2
Adaptation (n = 11)	401 ± 11	124 ± 8	76 ± 5	7.2 ± 0.2
Adaptation plus infarction (n = 11)	384 ± 13	94 ± 6	68 ± 4	5.5 ± 0.3

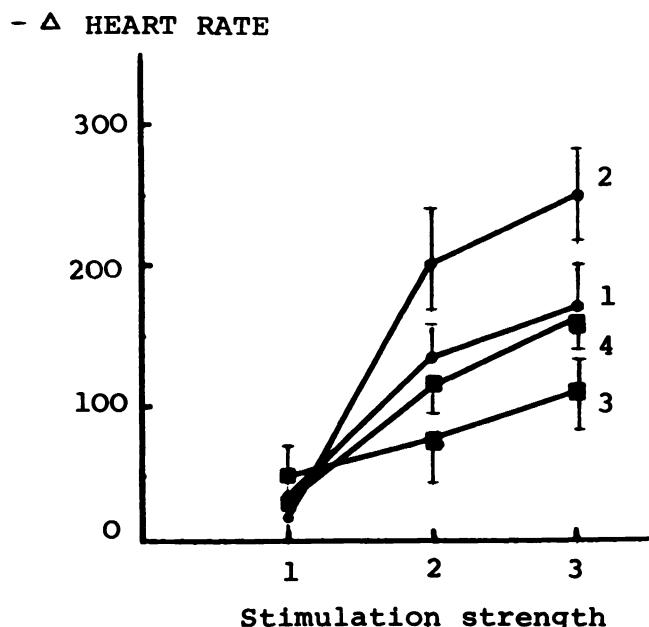


FIGURE 7. Effect of adaptation to stress on the negative chronotropic effect of vagal stimulation in acute myocardial infarction. The abscissa is strength of vagal stimulation, multiples of threshold current. The ordinate is the decrease in heart rate, min⁻¹. (1) Control, (2) infarction, (3) adaptation, (4) adaptation plus infarction.

Postinfarction cardiosclerosis did not appreciably change the heart rate, but in acute tests was consistently attended with the arterial systolic pressure lowered by 40 to 45 mmHg, i.e., more than by 25%. This pressure drop was not abolished by adaptation to stress. Further, postinfarction cardiosclerosis was consistently attended with a more than twofold decrease in the cardiac fibrillation threshold, which was completely abolished in adaptation.

Table 10 shows that cardiosclerosis increases somewhat the negatively chronotropic vagal effect, whereas adaptation to stress decreases it in both intact and cardiosclerotic animals by a factor of 1.5 to 2.3, thus more than completely overcoming the adverse influence of cardiosclerosis.

In these experiments, 30-s vagal stimulation could not elicit ectopic activity in adapted or nonadapted animals without cardiosclerosis. In nonadapted cardiosclerotic animals, extrasystoles are evoked already at threshold stimulation, with the cumulative number of extrasystoles over the four periods of stimulation (Table 11) amounting to 516, whereas adaptation to stress reduces this number more than threefold. This protective effect is certainly

TABLE 10
Effect of Adaptation to Short Stress Exposures on the Negative Chronotropic Effect (NCE) of the Vagus Nerve in Postinfarction Cardiosclerosis

Series ^a	Initial heart rate (min^{-1})	NCE (min^{-1}) ^b at vagal stimulation, threshold units		
		1	2	4
Control	412 \pm 9	38 \pm 6	105 \pm 20	151 \pm 20
Cardiosclerosis	398 \pm 12	40 \pm 7	130 \pm 20	194 \pm 20
Adaptation	401 \pm 9	33 \pm 3	47 \pm 9 ^c	105 \pm 14 ^c
Cardiosclerosis + adaptation	358 \pm 14	29 \pm 4	55 \pm 9 ^c	96 \pm 10 ^c

^a Each series comprised 11 animals.

^b Expressed as decrease in the heart rate (beats per minute).

^c $p < 0.05$ compared to the control.

TABLE 11
Effect of Adaptation to Short Stress Exposures on Cardiac Ectopic Activity During Vagal Bradycardia in Postinfarction Cardiosclerosis

	Cardiosclerosis (n = 11)	Cardiosclerosis plus adaptation (n = 11)
Total number of animals with extrasystoles	9	5
Number of extrasystoles in 30 s of vagal stimulation at:		
1 threshold	40	0
2 thresholds	65	1
3 thresholds	194	85
4 thresholds	262	80
Cumulative number of extrasystoles in group	561	166

Note: Control and adapted animals without cardiosclerosis had no extrasystoles during vagal stimulation in these experiments.

due to attenuated vagal action and probably to antiarrhythmic action of stress-limiting systems activated in adaptation.

Thus, *adaptation to stress abolishes the disturbances of cardiac electric stability (lowered fibrillation threshold and increased ectopic activity) characteristic of postinfarction cardiosclerosis.*

For appreciation of this result it is essential that the animals were taken into study 1 month after infarction. Hence, neither stress or ischemia could play any role in the observed disorders, and the beneficial effect of experimental therapy cannot be attributed to an anti-ischemic or antistress effect. The decreased fibrillation threshold and increased ectopic activity in postinfarction cardiosclerosis are due to the existence of a large enough connective-tissue scar in the left ventricular wall. It has been shown that in the scar and especially in its border zone there always are inclusions of cardiomyocytes that have survived acute ischemia and retain their bioelectric activity and near-normal histological structure; in recent studies these cells have been found to have lowered rest potential, decreased amplitude and upstroke velocity of the action potential (AP), and diversified alterations of the duration of AP and refractory phase.⁵⁸

A priori, this complex of alterations can be supposed to constitute the basis of irregular conduction of excitation and to be thus involved in formation of reentry and cardiac fibrillation

upon a premature impulse from an ectopic focus. In our experiments such an impulse was created by premature electric stimulation of the apex cordis, and the current necessary to cause reversible fibrillation in animals with postinfarction cardiosclerosis was half that in the control. The above interpretation is in complete accord with the results of clinicophysiological studies where the surviving cells of the border zone in postinfarction cardiosclerosis in humans were identified as the origin of ventricular tachycardia in chronic IHD patients.

In an epidemiological study of Wilhelmsson et al.,⁵⁹ people with rich stress experience had a reliably better life expectancy after myocardial infarction than people that had led a relatively peaceful life. Analogous data were earlier presented by Jenkins et al.,⁶⁰ who showed that people exhibiting a high emotional reaction to encountered situations have lower mortality from first and subsequent myocardial infarction than those with a moderate reaction. Of course, these clinical observations should be verified and extended. However, they are in line with the notion that rationally organized training to extremal situations such as the one acquired in military service lowers the probability of sudden cardiac failure.

On the whole, the body of facts available to date gives rise to at least two questions. First, can adaptation to small stresses be practically applied to heart protection?, and second, how does the phenomenon of adaptational protection change our theoretical notions of the mechanisms of arrhythmias and sudden death?

In solving the first of these questions, we tried to find out whether or not adaptation to such rough treatment as immobilization could be replaced with transauricular acupuncture, which is widely used on humans and is not associated with such great energy and structural expenditures as in the immobilization stress.

These studies⁶¹ were carried out on Wistar male rats weighing 200 to 250 g; the animals were grouped in six series: (1) control; (2) a course of electroacupuncture (EA); (3) a 12-h immobilization stress; (4) stress after EA; (5) acute ischemia in left coronary artery occlusion; and (6) acute ischemia after EA. For EA, needles were introduced into the floor of both auricles near the external acoustic meatus, and pointed impulses (0.8 to 2 mA, 1.5 ms, 3 Hz) were delivered with a Jasper CS-504 electrostimulator for 20 min daily for 2.5 weeks. This dosage of electrostimulation approximated those used for reflexotherapy in humans. The immobilization stress was produced by fixing the animals by four limbs in the supine position for 12 h. Acute ischemia in conscious, closed-chest animals was produced according to Lepran et al.⁴⁸ All physiological and biochemical techniques have been described earlier. Catecholamine content in the adrenergic fibers of right ventricular myocardium was assayed by the luminometric method of Folck-Owman as modified by Krokhina.⁶²

The transauricular EA used by us never elicited any pain reaction. Starting from the second or third exposure, the animals after 5 to 7 min of stimulation grew torpid and remained such for several minutes after the end of stimulation. During the seance the animals could be easily handled and laid on the back, i.e., there was no avoidance reflex or aggressive reaction.

The catecholamine content, phosphorylase A to total phosphorylase ratio, and glycogen content in the myocardium did not appreciably change after EA (Table 12), indicating the absence of a stress effect on the heart. The table further shows that immobilization stress, as in earlier works,⁶³ markedly decreased (by a factor of 2.5) the myocardial catecholamine content, with the ratio of active phosphorylase A to total phosphorylase increasing by half. Concurrent mobilization of glycogen decreased its myocardial content by one third. After the course of EA, which itself did not alter these indices, the effect of stress was different: the catecholamine content declined by not more than 25%, while the phosphorylase ratio and glycogen did not change significantly (Table 12).

The cardiac resistance to arrhythmogenic influences is known⁶⁴ to be inversely correlated with the phosphorylase ratio. The data of Table 13 agree with this nicely: the EA course as such only slightly raised the cardiac fibrillation threshold, but clearly attenuated its drop in

TABLE 12
**Effect of Electroacupuncture (EA) on Catecholamine Content,
 Phosphorylase Activity, and Glycogen Content in Rat Heart
 During Stress**

Series	Catecholamines, luminescence units	Phosphorylase A/(A + B) ratio	Glycogen (mg/ 100 g tissue)
Control	17.6 ± 1.57 (n = 5)	0.43 ± 0.03 (n = 7)	435 ± 14 (n = 7)
EA	17.21 ± 1.41 (n = 5)	0.45 ± 0.05 (n = 10)	409 ± 14 (n = 10)
Stress	7.0 ± 0.87 (n = 5)	0.61 ± 0.04 (n = 8)	290 ± 6 (n = 8)
	$p_{1-3} < 0.001$	$p_{1-3} < 0.01$	$p_{1-3} < 0.001$
EA + stress	13.7 ± 1.00 (n = 5)	0.47 ± 0.03 (n = 10)	398 ± 23 (n = 10)
	$p_{3-4} < 0.001$	$p_{3-4} < 0.01$	$p_{3-4} < 0.01$

TABLE 13
**Prevention of the Stress Decline in
 Myocardial Fibrillation Threshold with a
 Course of Electroacupuncture (EA)**

Series	Electric fibrillation threshold (mA)
Control (n = 10)	8.5 ± 0.5
EA (n = 9)	9.5 ± 1.5 $p_{1-2} \text{ NS}$
Stress (n = 10)	3.3 ± 1.0 $p_{1-3} < 0.001$
EA + stress (n = 8)	6.8 ± 0.8 $p_{3-4} < 0.001$

stress. In other words, the EA course substantially alleviated the predisposition to arrhythmias usually created by the immobilization stress. Table 14 shows the effect of EA on the development of cardiac arrhythmias within 10 min of coronary occlusion in conscious animals. After EA there is a certain tendency to an increase in the latent period of arrhythmias, a more than fivefold decrease in the duration of ventricular tachycardia, and a more than threefold decrease in the overall duration of arrhythmias (mean values per animal).

Thus, a course of EA producing no defense reactions in animals or stress effects on the heart prevents activation of phosphorylase, mobilization of glycogen, markedly attenuates the drop of the cardiac fibrillation threshold in stress, and also substantially restricts the duration of arrhythmias in acute ischemia. Since in acute ischemia in conscious animals stress plays an important part in the development of arrhythmias, the data obtained prompt an idea that the cardioprotective antiarrhythmic action of EA is mostly due to its stress-limiting effect, i.e., depends on reflex activation of the stress-limiting systems.

This suggestion is supported by the fact that acupuncture causes accumulation of the GABA-ergic and opioidergic metabolites in the brain.⁶⁵⁻⁶⁷ This makes promising further studies on the antiarrhythmic action of acupuncture in the clinic and on the stress-limiting systems as an important link in the therapeutic effect of this widely used method.

The adaptive activation of the stress-limiting systems and consequent adaptational protection of the heart against stress and ischemic damage and attendant impairment of cardiac electric stability, arrhythmias, and fibrillation are, in our opinion, important for further

TABLE 14
Effect of a Prior Course of Electroacupuncture (EA) on
Arrhythmias in Coronary Artery Occlusion

Indices (mean per animal)	(v) Control (n = 11)	(vi) EA (n = 10)	<i>p</i> _{v-vi}
Latent period of arrhythmias, seconds	70 ± 29	130 ± 40	
Number of extrasystoles	24 ± 12	27 ± 7	
Ventricular tachycardia, seconds	62.3 ± 23.7	11.8 ± 8.9	<0.01
Ventricular fibrillation, seconds	34.2 ± 8.5	26.6 ± 6.6	
Total duration, seconds	114 ± 38.5	52.8 ± 10.8	<0.05

development of our understanding of the pathogenesis of arrhythmias, fibrillation, and sudden death.

Indeed, we could have already seen that people with definitely proved absence of ischemic disease may severely suffer and even die from neurogenic arrhythmias and cardiac fibrillation of stress origin, and on the other hand such dramatic cases are only few in the trials of life.

Further, considering the data on the epidemiology and pathologic anatomy of ischemic disease, we could see that with the same degree of atherogenic coronary sclerosis people may live and people may die of fibrillation and sudden death. It should be noted that arrhythmias can often be triggered (and thus the question of life or death decided) by environmental, biosocial, stressful situations. Even after an infarction has already formed, the development of fibrillation and the fatal outcome can be determined by the extent of stress reaction and its cardiotropic effect.

The fact that adaptation to stress (providing activation of stress-limiting systems) completely prevented in our experiments the impairment of cardiac electric stability in stress and infarction, abolished these phenomena in postinfarction cardiosclerosis, and substantially alleviated grave arrhythmias and fibrillation in acute ischemia, taken together with clinical data prompts an idea that the hereditary or ontogenetically acquired state of the stress-limiting systems in great measure predetermines the absence or occurrence of arrhythmias and sudden death.

The concept of the relation of the stress-effecting and the stress-limiting systems in the pathogenesis of arrhythmias and fibrillation is to some extent reflected in the scheme of Figure 8. Under the influence of exogenous stress factors or afferent impulses from the ischemically damaged heart, in the higher divisions of the central nervous system (presumably in the frontal cortical lobes and in the adrenergic centers of the hypothalamus and medulla oblongata) there forms a stable enough system of excited centers which can once or repeatedly induce a strong adrenergic effect on the heart. This general layout is common for Schemes A and B, while their collation is an attempt to answer the eternal clinical question: why in most stress situations people have neither arrhythmias nor fibrillation, and furthermore, why fibrillation does not develop in most IHD patients and in most cases of acute ischemia? Figure 8A shows that such development of events is checked by activation of powerful stress-limiting systems, which is elaborately coupled with the stress reaction and prevents its injurious effects both at the central level (1) and in the heart proper (2). Clearly, such effective performance of the stress-limiting systems may be genetically determined or acquired through adaptation, as observed in our experiments. Figure 8B shows that such activation proves ineffectual in deficiency of the stress-limiting systems, either genetic or acquired because of prolonged stress exposure, intoxication, starvation, avitaminoses, etc. Then the cardiotropic detrimental effect of stress on the heart takes place by itself or combines with ischemia. It is such conditions that can most probably give rise to arrhythmias, fibrillation, and cardiac arrest.

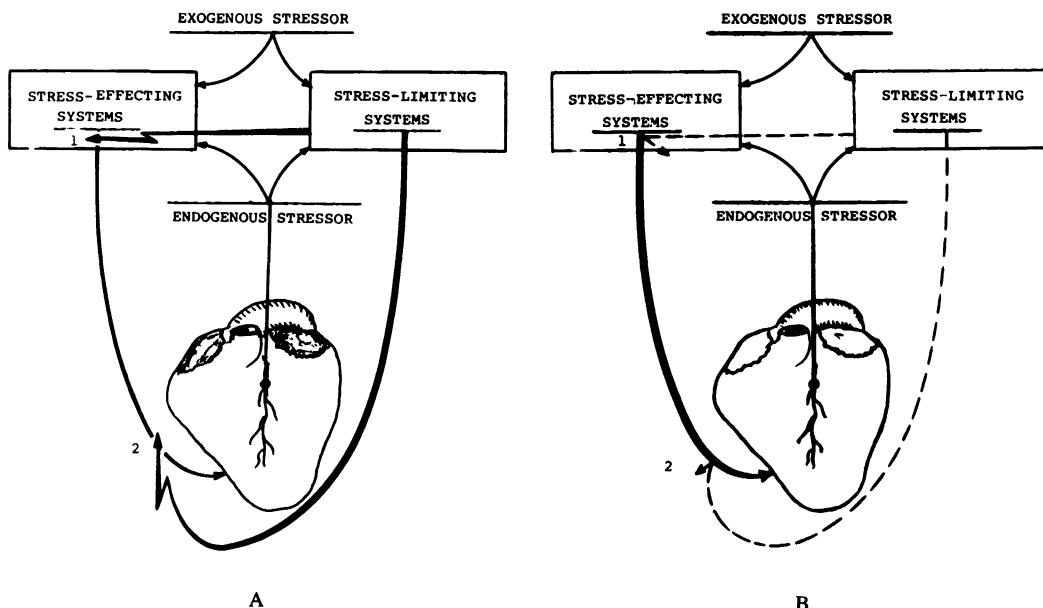


FIGURE 8. Adaptational increase in the physiological efficiency of stress-limiting systems prevents development of arrhythmias. (A) Organism with powerful stress-limiting systems. (B) Organism with deficient stress-limiting systems. (1) Central level of regulation; (2) cardiac self-regulation. For explanations see text.

It is easy to see that this working hypothesis is in accord with the real state of things presented in the preceding Chapter in detailed juxtaposition of stress arrhythmic heart disease and ischemic heart disease. Indeed, in stress arrhythmic disease the arrhythmias are clearly seen as neurogenically determined: they are readily elicited with stress load, but not with physical load, and develop despite intact coronary arteries in the absence of atherogenic dyslipidemia or coronary stenoses, i.e., in this case we are dealing with a selective pathology of the stress-limiting systems. Of course in this group there can also evolve secondary atherogenic dyslipidemia and coronary atherosclerosis owing to the above-considered atherogenic effect of stress.

In classical ischemic disease the situation is different. A primary defect in the cholesterol metabolism in the liver gives rise to atherogenic dyslipidemia with ensuing progressive coronary stenoses and ischemia which, depending on the state of the stress-limiting systems, may be aggravated by arrhythmias.

This is of course a most superficial description of the problem; to present a more specific concept of the stress-limiting systems, and to rationally use the mediators of these systems and their synthetic analogs as well as dosed adaptation for heart protection, a more concrete analysis is needed of the central and local stress-limiting systems and their place in the adaptational protection of the heart.

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Chapter 4

CENTRAL STRESS-LIMITING SYSTEMS AND CARDIOPROTECTIVE EFFECTS OF THEIR MEDIATORS AND ACTIVATORS

I. INTRODUCTION

Before considering the specialized modulatory stress-limiting systems of the organism, it should be noted that certain possibilities of self-restraint are inherent in the very mechanism of the stress reaction and its realization at the cell level. Table 1 reflects the results of our work¹ showing that upon 6-h emotional pain stress (EPS), besides the well-known shifts such as decreased content, synthesis, and neuronal uptake of norepinephrine, there is a considerable increase in the content of dopamine (almost threefold in the adrenal glands and 70% in the heart).

The fact that increased consumption and decreased content of norepinephrine and epinephrine in stress are consistently accompanied by dopamine accumulation in both organs can only be explained, within the present day concept of the catecholamine biosynthetic pathway, by the fact that in stress their formation is limited not in the first step (tyrosine hydroxylase which converts tyrosine to dopa), but at the dopamine β -hydroxylase step converting dopamine to norepinephrine. This would result in the events observed in our experiment: mobilization of the catecholamine reserve, combining with a significant elevation in dopamine in both the stress-effecting (adrenal glands) and the executive (heart) organs. In assessing the possible physiological role of dopamine accumulation in the response to a strong protracted exposure to stress, it should be borne in mind that dopamine, being a motor activator,² vasodilator, and natriuretic,³ participates in the adaptive effect of stress and at the same time exerts a regulatory influence on the stress reaction itself. Thus, dopamine inhibits pituitary secretion of ACTH⁴ and suppresses the ACTH-induced adrenal biosynthesis of corticosterone.⁵ It acts, therefore, as a factor restricting the hypophysial-adrenal link in the stress reaction.

Basically similar self-restraint is observed for stress hormones acting at the cell membrane level. One of the mechanisms of self-controlled cell activation in response to an excessive stress signal is associated with release of arachidonic acid. As already mentioned in Chapter 1, hydrolysis of cell membrane phospholipids caused by agonists of Ca^{2+} -mobilizing receptors, besides diacylglycerol (DAG) and inositol triphosphate (IP_3), produces arachidonic acid; it gives rise to prostaglandins E_1 , E_2 , and I_2 , which can effectively restrict cell activation by adrenergic stimuli through suppressing norepinephrine "discharge" from sympathetic terminals.⁶

Self-restraint mechanisms operate at the intracellular level as well; an example is stimulation of the cAMP phosphodiesterase by calmodulin,⁷ which checks cAMP elevation and thereby cell activation by catecholamines and other hormones acting on the adenylate cyclase and the cAMP circuit.

Thus, into the design of the stress reaction the evolution has built such features as allow the developing reaction to restrict itself. However, this does not appear to completely solve the problem, and accordingly the stress reaction is modulated by specialized stress-limiting systems. We shall consider three important examples, namely the GABA-ergic system and the linked system of benzodiazepine receptors; the opioidergic system and linked serotonin mechanisms; and parasympathetic neural regulation of the heart, whose role in restricting the arrhythmogenic adrenergic effects is known, but turns out to need further study.

TABLE 1
Effect of a 6-h EPS on Neuronal Uptake of ^3H -Norepinephrine, ^3H -Catecholamine Synthesis ($10^3 \text{ cpm/g tissue}$), and Catecholamine Content ($\mu\text{g/g}$) in the Rat Heart and Adrenal Glands

Indices	Heart		Adrenal glands	
	Control	EPS	Control	EPS
Neuronal uptake of ^3H -norepinephrine	70.7 \pm 4.5	48.6 \pm 3.5 ^a	53.9 \pm 2.7	41.2 \pm 3.9 ^b
Synthesis of ^3H -norepinephrine	7.4 \pm 0.5	5.2 \pm 0.6 ^b	7.2 \pm 0.6	5.3 \pm 0.2 ^b
Synthesis of ^3H -dopamine	1.9 \pm 0.08	2.2 \pm 0.26	4.3 \pm 0.32	4.3 \pm 0.54
Content of norepinephrine	0.99 \pm 0.19	0.56 \pm 0.10 ^c	213 \pm 26	149 \pm 15 ^c
Content of epinephrine	—	—	402 \pm 39	209 \pm 23 ^a
Content of dopamine	0.1 \pm 0.01	0.17 \pm 0.01 ^c	2.51 \pm 0.3	7.10 \pm 0.8 ^a

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

II. GABA-ERGIC SYSTEM AND BENZODIAZEPINE RECEPTORS*

At present the GABA system is regarded as the main regulatory checking system of mostly central action. On the other hand, in recent years it has been ascertained that besides the central nervous system (CNS, where the GABA system is represented by GABA-ergic neurons) GABA, enzymes of its synthesis and breakdown (respectively, glutamate decarboxylase [GDC] and GABA-transaminase [GABA-TA]), and its receptors are found in almost all organs and tissues^{8,9} where GABA is involved as a modulator of their neurohumoral regulation.⁹

For us it is essential that the GABA-ergic system is well represented at all levels of cardiac regulation: in the frontal cortex^{8,10} where there are neurons responsible for activation of sympathetic and parasympathetic cardiac regulation in stress;^{11,12} in the hypothalamic vegetative centers^{13,14} forming the common efferent "outlet" to the heart;¹⁵ in the nuclei of the brain stem parasympathetic cardiovascular center;¹⁶ in the cardiac afferentation center, i.e., nucleus tractus solitarius of the medulla oblongata;¹⁷ in the cardiac sympathetic nuclei of the spinal cord;¹⁸ and finally in the heart itself.⁹ Such full representation of the GABA system in heart and its regulatory apparatus points to the indubitable importance of GABA in control of cardiac function. This has indeed been proved in the works of recent years that will be considered later.

The coupling of the GABA-ergic system with the stress-effecting system manifests itself first of all as GABA elevation in stress in various brain regions: frontal cortex, hypothalamus, striatum, thalamus,^{10,19} increased glutamate formation and GABA content in brain hemispheres,²⁰ etc.; the excess of catecholamines²¹ and corticosteroids²² arising in the stress reaction depresses the activity of GABA-TA, the breakdown enzyme, entailing a rise in brain GABA content. In EPS, GABA increases in just those brain regions where GABA-TA declines.¹⁰ The coupling between the GABA and the stress-effecting system also reveals itself in direct activation of the GABA neurons by catecholamines released in the brain adrenergic structures in the stress reaction. Direct evidence is available for activation of the GABA system in the cortex and hypothalamus by norepinephrine and electrostimulation of adrenergic neurons.^{19,23}

Such activation of the GABA-ergic system can in its turn restrict the activity of the

* With M. G. Pshennikova.

stress-effecting systems and the stress reaction itself through negative feedback. This stress-limiting function of GABA appears to be performed by two basic mechanisms.

First, in the hypothalamus GABA has been found to restrict at the postsynaptic level the secretion of the ACTH-releasing factor and thereby the activation of the adrenal-hypophysial link of the stress reaction.^{24,25} Further, GABA exerts a tonic inhibitory influence on the population of "command" neurons in the hypothalamus. This population, when activated, may generate an integrative multisystem response consistently found in emotional stress, i.e., enhanced vegetative organ function, behavioral reactions, etc., constituting the stress reaction (see Reference 13 for references). Thus, activation of the GABA system can restrict the stress reaction at the central level.

Second, at the presynaptic level GABA can attenuate norepinephrine release from sympathetic terminals that innervate organs and tissues, and thereby restrict adrenergic activation both in the brain and in the target organs.^{6,26}

The above is schematically presented in Figure 1. In accordance with the abundance of the GABA system at all levels of cardiac regulation, in recent years it has been shown that the GABA-ergic neurons exert a tonic inhibitory influence on the brain centers regulating cardiac and vascular function, and cessation or weakening of this influence results in altered heart rate, hypertension, and cardiac rhythm disturbances. Thus, blocking the GABA receptors with bicuculline and picrotoxin or deficit of GABA induced with 3-mercaptopropionic acid lead to activation of the posterior hypothalamic centers of sympathetic control of the cardiovascular system^{13,14} with ensuing tachycardia, hypertension, tachyarrhythmias, and extrasystole. Administration of a GABA_A-receptor agonist muscimol or a β-adrenoblocker propranolol prevents these events.^{13,14,27} The GABA neurons also display such tonic inhibitory influence on the cardiac parasympathetic regulation centers of the nucleus ambiguus of the brain stem. Cessation of this influence entails bradycardia and bradyarrhythmias that can be abolished by dissection of the vagus nerve.^{16,27}

GABA has also been recently shown to block the afferent flow of pain impulses including the one from ischemic myocardium,²⁸ which can prevent formation of an excitation focus in the large hemisphere cortex responsible for cardiac arrhythmias.¹¹

Of special interest for us is the influence of the GABA system on the hypothalamic centers of cardiac sympathetic regulation, since it is these centers that are responsible for activation of the cardiac function in the stress reaction. In particular, it is in the posterior hypothalamus that the local blood flow is enhanced in response to emotional stress, indicating enhanced metabolic activity resulting from local neuronal excitation.²⁹ Destruction of this zone in baboons eliminates the cardiovascular response to acute emotional stress.³⁰

Taken together with the data on the role of GABA in suppression of the hypophysial-adrenal link of the stress reaction, the available facts give grounds for supposing that *GABA deficiency caused by hereditary defects of mediator turnover, brain tissue injury, or other factors can impair the cardiac and vascular regulation and potentiate the stress reaction and its detrimental action on the cardiovascular system; conversely, augmented capacity of the GABA system can prevent such damage.*

Indeed, spontaneously hypertensive rats have been shown to display increased activity of the sympathetic nerves which is normalized upon administration of the GABA_A-receptor agonist muscimol into brain ventricles,³¹ and to have congenital deficiency of GABA-ergic activity in the posterior hypothalamus,³² i.e., in the zone where GABA exerts its tonic inhibition of the centers of sympathetic regulation of vascular tone. Thus, hypertension in these animals can be thought to result from enhanced activity of vascular sympathetic regulation because of the lack of GABA-ergic inhibition.^{14,29}

On the other hand, adaptation to stress exposures, which restrict activation of stress-effecting systems and the detrimental effects of the stress reaction (see the preceding chapter), is accompanied with an increase in the functional capacity of the GABA-ergic system.^{33,34}

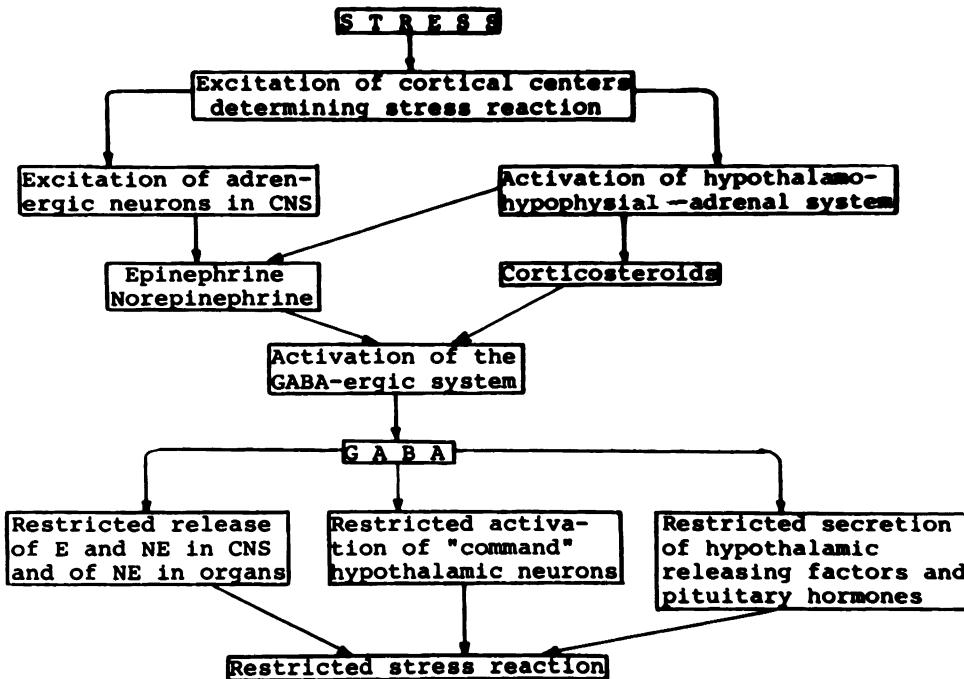


FIGURE 1. Coupling of the GABA-ergic system with the stress-effecting systems, and GABA-mediated restriction of the stress reaction. For explanations see text.

Thus, our studies show that in adaptation of rats to short immobilization stresses the activity of GDC in the large cerebral hemispheres increases while the GABA-TA activity remains the same, and accordingly the GABA content rises almost by 70%.³³

This agrees with the recent data that adaptation of rats to repeated immobilization stress activates the GABA system in the frontal cortex as manifest by increased GDC activity and GABA turnover in this brain region.³⁴ The authors emphasize that activation of the GABA system in adapted animals combines with attenuated function of the peripheral sympathetic system which is controlled by GABA-inhibitable sympathetic centers.³⁴ All these data are also in line with the known facts that adaptation to repeated stress enhances animal resistance to the convulsive action of electric current and GABA antagonists,³⁵ and that administration of GABA or its active metabolite GHBA suppresses the stress reaction and prevents its detrimental effect.³⁶

On the whole, the above gave grounds for using activators of the GABA system and its active metabolite GHBA to prevent cardiac stress damage as well as diseases in which the stress reaction is pathogenetically important, and first of all stress-induced and ischemic arrhythmias.

A previously published monograph³⁷ presented the data from our laboratory that GHBA can restrict the stress reaction as judged by blood corticosterone and simultaneously prevent such manifestations of stress damage as enzymemia, ulcerous lesions of the gastric mucosa, enhanced myocardial lipid peroxidation, and impaired cardiac contractile function. These data are in accord with clinical observations on the use of GHBA as an antiarrhythmic. Here we shall consider new material on the cardioprotective action of the GABA-ergic activator sodium valproate, which consistently raises the GABA content in the brain³⁸ and in the blood,³⁹ but has not heretofore been applied in cardiology.

At the first stage of this work⁴⁰ we studied the effect of sodium valproate on arrhythmias attending acute cardiac ischemia produced in conscious animals according to Lepran et al.⁴¹

TABLE 2
Effect of Sodium Valproate on Cardiac Rhythm Disturbances in Acutely Ischemic Conscious Rats

	Control (n = 20)	Valproate (n = 20)
Extrasystole		
Number of animals	12	6
Total duration in group	211	83
Mean duration per animal	10.6 ± 9.6	4.2 ± 5.8
Ventricular tachycardia		
Number of animals	13	6
Total duration in group	394	122
Mean duration per animal	19.7 ± 17.3	6.1 ± 6.4
Ventricular fibrillation		
Number of animals	12	2
Total duration in group	562	56
Mean duration per animal	28.1 ± 23.7	2.8 ± 5.0
All arrhythmias		
Number of animals	15	6
Total duration in group	1107	261
Mean duration per animal	55.4 ± 36.4	13.1 ± 15.2
Deaths	7	0

The study was carried out on 40 male Wistar rats weighing 230 to 280 g; half of the animals received 200 mg valproate per kilogram *per os* 90 min before acute ischemia, and the other 20 served as controls. Table 2 shows that acute ischemia was attended with grave arrhythmias in most of the controls: 13 animals (65%) developed ventricular tachycardia, and 12 animals (60%) ventricular fibrillation with a lethal outcome in 7 cases. In the group that had received sodium valproate the incidence of all types of arrhythmia, extrasystole, and ventricular tachycardia decreased by a factor of 2 to 2.5. The incidence of fibrillation fell sixfold and, most importantly, deaths were completely averted. It should also be noted that the overall duration of all types of arrhythmia decreased more than fourfold (ventricular fibrillation tenfold).

Thus, we can see that the antiarrhythmic effect of adaptation in acute myocardial ischemia in conscious animals can be efficiently mimicked by activating one of the main stress-limiting systems of the organism, the GABA-ergic one.

Next, experiments were run on anesthetized rats to assess the effect of sodium valproate on the drop of ventricular fibrillation threshold in acute ischemia and on the extent of ischemic arrhythmias. Before acute ischemia, the fibrillation thresholds were about the same in both groups: 10.8 ± 1.1 mA in the control and 11.7 ± 0.8 mA with valproate. Acute ischemia drastically decreased the fibrillation threshold in the control group: the drop was thirteenfold ($p < 0.01$), whereas after valproate administration the decrease was less so that the ischemic threshold (2.5 mA) was still three times higher than in the control ($p < 0.01$). It is also noteworthy that acute ischemia resulted in a substantial lengthening of the QT interval ($p < 0.01$), which reflects the onset of the vulnerable period.⁴² This can probably be explained by depression of the rest potential (RP) of cardiac cells in acute ischemia,⁴³ which results in decreased cell excitability and longer refractory state.⁴⁴ Sodium valproate not only lessened the drop of the fibrillation threshold, but also substantially prevented the changes in QT, i.e., most likely restricted the depression of the RP and associated alterations in cell excitability.

Thus, prior administration of the GABA-ergic activator sodium valproate significantly decreased the probability of fibrillation in acute myocardial ischemia.

Next, the effect of sodium valproate was studied on arrhythmias developing in acute ischemia imposed with left coronary artery ligation in anesthetized animals. These experi-

ments confirmed the well-known fact that anesthesia substantially restricts the stress reaction and thereby decreases the overall extent of arrhythmia in cardiac ischemia as compared to conscious animals. Nevertheless, even under such conditions the antiarrhythmic effect of sodium valproate proved definite enough. Thus, in the control all animals had extrasystoles and ventricular tachycardia, and eight out of ten developed cardiac fibrillation which, however, was reversible. Prior administration of sodium valproate appreciably diminished the incidence of fibrillation and decreased the total duration of all types of arrhythmia threefold ($p < 0.01$), decreased the duration of extrasystole by about 25%, ventricular tachycardia by 30%, and fibrillation sevenfold. There was also a tendency to a decrease in the mean duration of different types of arrhythmia. *So, the antiarrhythmic effect of valproate in acutely ischemic anesthetized rats was similar to the one in conscious animals.*

The second stage of our work was aimed at assessing the influence of sodium valproate on cardiac electric stability in experimental myocardial infarction. The studies were carried out with rats 48 h after coronary artery occlusion according to Selye. The acute tests were performed under anesthesia. The cardiac fibrillation threshold and ectopic activity were determined as described in Chapter 2. Sodium valproate did not appreciably change the heart rate (Table 3), but largely prevented the drop of the fibrillation threshold in infarction and practically eliminated the extrasystoles commonly observed with vagal stimulation in infarction.

Thus, the GABA-ergic activator prevents profound impairment of cardiac electric stability typical of myocardial infarction.

Since many antiarrhythmics are known to depress cardiac contractile function, it was important to find out the “costs” of this effect. Table 4 shows that *valproate did not additionally depress the parameters of cardiac contractile function in experimental myocardial infarction. In essence, the above experiments were aimed at prevention of cardiac arrhythmias in ischemic damage, i.e., represented experimental prophylaxis.*

At the third and final stage, we tried to use sodium valproate for experimental therapy of cardiac electric stability disorders in postinfarction cardiosclerosis, 2 months after experimental myocardial infarction. These experiments were carried out with larger rats (350 to 400 g) who therefore had a lower heart rate under anesthesia. The results are presented in Table 5 and are quite similar to those obtained in infarction.

In the aggregate, the beneficial antiarrhythmic effects of sodium valproate in acute ischemia in both conscious and anesthetized animals, in myocardial infarction, and in postinfarction cardiosclerosis make most topical a study of the antiarrhythmic action of such GABA-ergic activators in clinical cardiology.

It is now known that the GABA system is regulated, besides other mechanisms, by benzodiazepine receptors localized in the synaptic structures of the CNS and allosterically coupled with the postsynaptic GABA_A receptors.^{8,45} The main function of the classical ligands of benzodiazepine receptors — 1,4-benzodiazepines — is potentiation of the GABA relay at all CNS levels, actualized in the form of anticonvulsive, anxiolytic, and hypnotic efforts.^{45,46} Antagonists and inverse agonists of these receptors, on the contrary, weaken the inhibitory action of GABA and accordingly aggravate convulsions, fear, excitement, etc.⁴⁵ Benzodiazepines act allosterically on the GABA receptor to potentiate the GABA-induced opening of the chloride channel and chloride entry into the neuron.^{45,46} It can thus be supposed that benzodiazepines enhance the above mentioned GABA-ergic tonic inhibition of the hypothalamic and brain stem neurons realizing the sympathetic influence on the heart, vessels, and other organs,^{13,14,29} and can thereby restrict activation of these neurons in stress. In particular, a benzodiazepine diazepam (Valium®) has been shown effective in preventing stress-induced hypertension.⁴⁷ Benzodiazepines probably also augment the checking effect of GABA in the cortex, which is rich in benzodiazepine receptors.⁸

At present there is no doubt that activation of the benzodiazepine receptors relieves emotional tension and excessive psychomotor reactivity in stress. Basing on this, we used

TABLE 3
Effect of Sodium Valproate on the Cardiac Fibrillation Threshold and Ectopic Activity in Myocardial Infarction

Series	Heart rate (min ⁻¹)	Fibrillation threshold (mA)	Total number of extrasystoles in vagal bradycardia
Control (n = 8)	400 ± 19	6.4 ± 0.37	0
Infarction (n = 8)	438 ± 18	2.6 ± 0.51 ^a	103
Valproate (n = 8)	412 ± 13	6.8 ± 0.42	0
Infarction plus valproate (n = 8)	422 ± 17	5.5 ± 0.71 ^b	2

^a $p_{1-2} < 0.01$.

^b $p_{4-2} < 0.01$.

TABLE 4
Effect of Sodium Valproate on the Left Ventricular Contractile Function 48 h After Myocardial Infarction

Series	Heart rate (min ⁻¹)	Developed pressure (mmHg)	IFS
Control (n = 10)	448 ± 4	118 ± 4.4	87.2 ± 4.7
Infarction (n = 10)	407 ± 18	87 ± 3.9 ^a	66.7 ± 4.1 ^a
Valproate (n = 11)	441 ± 6	119 ± 2.8	93.1 ± 4.4
Infarction plus valproate (n = 9)	381 ± 16	79 ± 2.2 ^b	62.0 ± 4.2 ^b

^a $p_{1-2} < 0.01$.

^b $p_{3-4} < 0.01$.

TABLE 5
Normalization of Cardiac Electric Stability in Postinfarction Cardiosclerosis With Sodium Valproate

Series	Heart rate (min ⁻¹)	Fibrillation threshold (mA)	Total number of extrasystoles in vagal bradycardia
Control (n = 11)	389 ± 11	6.8 ± 0.7	19
Cardiosclerosis (n = 11)	379 ± 8	2.3 ± 0.3 ^a	401
Control plus valproate (n = 11)	380 ± 13	6.4 ± 0.7	0
Cardiosclerosis plus valproate (n = 11)	380 ± 12	6.3 ± 0.3	40

* $p < 0.01$.

a known benzodiazepine receptor agonist, phenazepam, to restrict ischemic and reperfusion arrhythmias and disturbances to cardiac electric stability in infarction and postinfarction cardiosclerosis.

Phenazepam is well known as a psychotropic and anticonvulsive drug. Therefore, we first studied the antiarrhythmic action of phenazepam in the above conditions and then briefly collated the antiarrhythmic and the anticonvulsive properties of this benzodiazepine receptor agonist.

Table 6 demonstrates the effect of phenazepam (1 mg/kg intraperitoneally, 1 h before ischemia) in acute ischemia and subsequent reperfusion. The total duration of all types of cardiac rhythm disorders was much less than in controls; the mean duration of all arrhythmias per animal decreased more than twofold in ischemia and almost fivefold in reperfusion. It is noteworthy that both the incidence and the mean duration of a grave form of arrhythmia

TABLE 6
**Effect of Phenazepam on Cardiac Rhythm Disorders in Acute
 Ischemia and Reperfusion in the Whole Organism**

Indices	Ischemia		Reperfusion	
	Control (n = 12)	Phenazepam (n = 12)	Control (n = 12)	Phenazepam (n = 12)
Extrasystole				
Number of animals	12	12	12	10
Total duration	395	287	76	49
Mean duration	33 ± 4	24 ± 3	6 ± 2	4 ± 2
Ventricular tachycardia				
Number of animals	12	10	12	11
Total duration	509	162	572	139
Mean duration	43 ± 9	14 ± 3*	48 ± 9	12 ± 4*
Ventricular fibrillation				
Number of animals	8	5	7	2
Total duration	203	18	272	6
Mean duration	17 ± 7	1.5 ± 2*	23 ± 15	0.5 ± 1*
All arrhythmias				
Total duration	1107	467	920	194
Mean duration	92 ± 19	39 ± 6*	77 ± 15	16 ± 6*

* Difference from respective controls significant at $p < 0.01$.

TABLE 7
**Effect of Phenazepam on Cardiac Fibrillation Threshold and Vagally Elicited
 Ectopic Activity in Acute Myocardial Infarction**

Series (n = 10 each)	Initial heart rate	Total no. of extrasystoles	Mean no. of extrasystoles	Fibrillation threshold (mA)
Control	393 ± 12	2	0.2	6.3 ± 0.5
Phenazepam	402 ± 9	0	0	6.9 ± 0.2
Infarction	403 ± 16	325	32.5 ± 12	1.5 ± 0.2
Infarction plus Phenazepam	398 ± 18	59	5.9 ± 5	4.0 ± 0.5
			<0.05	<0.001

— ventricular fibrillation — were markedly lower in both conditions after phenazepam administration. Thus, it has been proved that in the whole organism phenazepam has a pronounced antiarrhythmic effect in acute ischemia and reperfusion of the heart. Notably, this drug was ineffective in analogous experiments with isolated heart; i.e., unlike sodium valproate, it acted exclusively at the neural regulation.

The data in Table 7 reflect the results of phenazepam injection in acute myocardial infarction. The drug (1 mg/kg) was injected twice: before coronary occlusion and 1 h before testing the electric stability.

The disturbances of cardiac electric stability in unprotected animals were typical of acute myocardial infarction: enhanced suppression of the sinus node automatism upon vagal stimulation, numerous extrasystoles evolving at this background, and a fourfold drop of the electric fibrillation threshold. Phenazepam itself did not appreciably affect these parameters in normal animals, but largely attenuated the above disturbances in myocardial infarction (see Table 7).

To appreciate the real significance of this protective effect of phenazepam, one should consider the data on mortality from myocardial infarction in our experiments. In total, to

TABLE 8
Effect of Phenazepam on Cardiac Fibrillation Threshold and Vagally Elicited Ectopic Activity in Postinfarction Cardiosclerosis

Series (n = 9 each)	Initial heart rate	Total no. of extrasystoles	Mean no. of extrasystoles	Fibrillation threshold
Control	367 ± 11	5	0.5	5.5 ± 0.3
Phenazepam	347 ± 10	0	0	6.4 ± 0.2
Cardiosclerosis	352 ± 10	249	28 ± 14	2.0 ± 0.2
Cardiosclerosis plus phenazepam	338 ± 10	15	2 ± 2	4.0 ± 0.3
<i>P</i> ₃₋₄			<0.01	<0.001

produce acute infarction and postinfarction cardiosclerosis, coronary occlusion was performed in 70 rats without any protection; the first-day mortality was 47%. In 20 animals coronary occlusion was performed after single administration of phenazepam in the above dosage; only one animal died within the first day. Thus, in our hands phenazepam, by improving the cardiac electric stability in the acute period of experimental myocardial infarction, markedly decreased mortality.

Table 8 shows the results of experimental therapy of the disturbances of cardiac electric stability in postinfarction cardiosclerosis with phenazepam injected at 1 mg/kg intraperitoneally three times in 2 d prior to testing, the last injection 1 h before assaying the electric stability. As follows from Table 8, postinfarction cardiosclerosis was attended with pronounced ectopic activity of the heart; in vagal bradycardia there were numerous ventricular extrasystoles. The electric fibrillation threshold was lowered threefold. These data are in accord with the clinical observations of the high probability of arrhythmias and fibrillation in patients with postinfarction cardiosclerosis. Phenazepam practically completely abolished ventricular extrasystoles in vagal bradycardia (i.e., suppressed the ectopic activity of the heart) and doubled the fibrillation threshold (i.e., diminished the probability of fibrillation).

It is quite essential that the antiarrhythmic effect in acute myocardial infarction and postinfarction cardiosclerosis was achieved with doses of phenazepam that did not adversely affect arterial pressure. Indeed, Table 9 shows that in the absence of stress (in control and in postinfarction cardiosclerosis) the drug had no effect at all on the pressure in the carotid artery; in acute myocardial infarction the pressure in phenazepam-treated animals proved higher than in unprotected ones. This agrees nicely with prevention of mortality in experimental infarction shown above.

In the aggregate, *the experimental results testify that the benzodiazepine receptor agonist phenazepam to a large extent abolishes the disturbances of cardiac electric stability both in acute experimental myocardial infarction and in chronic postinfarction cardiosclerosis, diminishing in both cases the probability of ventricular arrhythmias and cardiac fibrillation.*

These facts are in accord with the results of another work⁴⁵ demonstrating a direct antiarrhythmic effect of this drug in acute ischemia and reperfusion, which takes place in the whole organism, but not in isolated heart and is therefore central.

In interpreting the antiarrhythmic action of phenazepam, it should be remembered that benzodiazepine receptors widely represented in the synaptic structures of the cortex⁸ and other brain regions are important for potentiation of the GABA effects at all CNS levels;^{45,46} on the other hand, activation of the central adrenergic mechanisms is decisive in impairment of cardiac electric stability in acute ischemia, and accordingly ischemic arrhythmias can be prevented with intracisternal administration of a β-blocker⁴⁸ as well as removal of the stellate ganglion.⁴⁹ Therefore, diazepines including phenazepam can be thought to boost the GABAergic tonic inhibition of the neurons exerting the adrenergic influence on the heart. As a result, excessive activation of these neurons usual in response to acute cardiac ischemia is checked upon administration of phenazepam, producing at the central level the above-described antiarrhythmic effect.

TABLE 9
Effect of Phenazepam on Blood Pressure In the Carotid Artery in Acute Myocardial Infarction and Postinfarction Cardiosclerosis

Series	Blood pressure (mmHg)	
	Systolic	Diastolic
Control (n = 10)	132 ± 4	108 ± 3
Phenazepam (n = 10)	128 ± 12	97 ± 12
Infarction (n = 10)	84 ± 5	71 ± 6
Infarction plus phenazepam (n = 10)	104 ± 3 ^a	86 ± 6
Cardiosclerosis (n = 9)	107 ± 5	97 ± 4
Cardiosclerosis plus phenazepam (n = 9)	106 ± 5	94 ± 5

^a $p_{3-4} < 0.01$.

Thus, the data obtained point out that benzodiazepine receptor agonists, as well as other GABA activators, in addition to their conventional psychotropic action display pronounced antiarrhythmic activity and deserve thorough clinical study.

By and large, it can be stated that *the concept of the GABA-ergic system is quite important for cardiology, since deficiency of this or the benzodiazepine receptor system can be involved not only in epilepsy, but in arrhythmias as well*, and pharmacological activation of the GABA-ergic system, either direct or through benzodiazepine receptors, is a promising approach to treating arrhythmias.

III. OPIOIDERIC SYSTEM*

The opioid peptides (OPs) were initially regarded as endogenous analgetic factors; it was just this action, similar to that of morphine preparations, that gave them their name (i.e., morphine-like). Now, however, there is no doubt that OPs are in fact regulators of numerous processes while their analgetic effect is only one manifestation of their modulatory function. The OPs and their receptors, i.e., both components of the opioideric system, have been found in the cells of different brain and spinal cord regions,^{50,51} peripheral divisions of the nervous system,^{50,52} as well as in adrenal glands⁵³ and other organs, including the heart.⁶

The coupling between the opioideric and the stress-effecting systems is well studied, and it is known that the opioideric system can directly participate in the stress reaction as a regulator of sensitivity to pain and as a modulator of emotional, behavioral, and other components of the reaction. This is based on two groups of experimental data. First, during the stress reaction production and liberation of OPs is stimulated in various brain regions, and their blood level rises. This is accompanied with such defensive reactions as elevation of the pain threshold and analgesia,⁵⁴ altered behavior,⁵⁵ hyperthermia,⁵⁶ etc., these reactions being prevented by opiate receptor blockers like naloxone or by opiate synthesis inhibitors. It must be mentioned that in electro- and acupuncture (which can be regarded as brief noninjurious stresses), the analgesia is also accompanied with OP elevation in the blood and in the cerebrospinal fluid, and inhibited with naloxone.⁵⁷ Second, administration of OPs or their synthetic analogs produces a dose-dependent analgetic effect and other defensive reactions observed in stress.

The coupling of the opioideric system to the stress-effecting one, which determines its activation in stress, can be ensured by genetic programming as well as by feedback. An

* With M. G. Pshennikova.

example of genetic coupling is the equimolar release at ACTH and beta-endorphin from the anterior pituitary body,^{58,59} which is due to ACTH and beta-endorphin being produced from the common polypeptide precursor pro-opiomelanocortin by the Leu-Phe peptidase.^{59,60} Analogously, coupled release of catecholamines and enkephalins from the adrenal glands⁶² is due to their common localization in the chromaffin vesicles.⁵³ A manifestation of feedback is the suppression of both ACTH and beta-endorphin production in the anterior pituitary body through inhibition of the Leu-Phe endopeptidase glucocorticoids released in response to ACTH.⁶⁰ The coupling between opioidergic and the stress-effecting systems appears to be manifold, and is reflected, in particular, in that the systems are intimately associated topologically and functionally both in the CNS and in the periphery.^{52,63,64}

With regard to the problem of cardiac neural regulation and cardiac arrhythmias, it is noteworthy that the thoracic segments of the spinal cord harbor cholinergic neurons whose axons innervate the sympathetic ganglia, being preganglionic, and the adrenal medulla, ending there on the chromaffin cells. At least a substantial portion of these neurons have been found to contain in their axonic vesicles both acetylcholine and enkephalins.⁵² Therefore, when these spinal neurons are activated their axons, besides acetylcholine (stimulating the sympathetic cells of the ganglia and release of adrenal catecholamines), also liberate enkephalins which can inhibit the release of acetylcholine and thereby restrict the neurotransmission in the sympathetic ganglia.⁶⁵

For understanding the direct stress-limiting effect of OPs, it is important that they are secreted substances accumulating biosynthetically in secretory granules or vesicles in the nerve endings or secretory cells. Their release is not constitutive, but is triggered by certain physiological conditions and signals, such as acute changes in the activity of the neurohormonal system, which is definitive for their modulatory function. Thus it has been found that, being localized in the nerve terminals together with norepinephrine, OPs are not released during moderate impulse activity of the nerve, i.e., during moderate liberation of the mediator; only in high and prolonged neural impulsation typical of stress reaction, they are released to inhibit further liberation of the mediator and to prevent its excessive activity.⁶³

Thus, the coupling between the opioidergic and the stress-effecting systems evolved in such a way that opioids are released timely and economically, only in a great or excessive adrenergic stress reaction.

This means that in the course of adaptation to stress the increasing power of the opioidergic system should reveal itself as direct accumulation of OPs in the corresponding brain and adrenal structures. Only this would allow the adapted animals to check the excitation evoked by adrenergic and cholinergic agents in the brain structures such as the frontal cortex, which has been pointed out as the probable site of formation of an arrhythmogenic excitation focus in acute cardiac ischemia.¹¹

Our studies have shown that OPs are indeed accumulated during adaptation to stress, and the catecholamine- or acetylcholine-induced excitation of the frontocortical neurons is indeed suppressed. Thus, using a radioimmunoassay technique we have been able to demonstrate⁶⁶ that adaptation to short stress exposures increases the concentrations of enkephalins and beta-endorphin in all brain structures examined and in the adrenal glands (Table 10). Clearly, a result of such adaptation would be that in stress or just upon a strong enough stimulation of the brain neurons the coupled release of OPs (and maybe mediators of other stress-limiting systems) would be enhanced and consequently the adrenoresponsiveness or cholinoresponsiveness of the neurons could be attenuated. To assess the reality of such attenuation at the brain-cortical level, we studied the effect of adaptation to short stresses on the chemoreactive properties of the neurons of the sensomotor cortex, namely on their sensitivity to acetylcholine and norepinephrine.⁶⁷ To this end, animals were paralyzed with alpha-tubocurarine, and with the use of a micromanipulator fastened to the cranium a three-bore microelectrode was submerged to the level of the IV-V layer of the cerebral

TABLE 10
Opioid Peptide Content in Brain Structures and Adrenal Glands
in Adaptation to Short Stress Exposures

	Peptide content (pmol/mg tissue)		
	Met-enkephalin	Leu-enkephalin	β -Endorphin
Control (n = 7)			
Cerebellum	22.3 ± 7.8	20.2 ± 3.8	
Hypothalamus	226.7 ± 9.2	123.3 ± 22.9	10.2 ± 0.8
Striatum	263.2 ± 8.9	186.0 ± 17.5	
Cortex	60.5 ± 7.5	49.1 ± 5.7	
Adrenal glands	53.7 ± 1.7	71.8 ± 8.4	
Adaptation (n = 7)			
Cerebellum	40.9 ± 7.1 ^a	37.2 ± 6.8 ^a	
Hypothalamus	422.3 ± 45.4 ^b	271.9 ± 41.2 ^b	17.8 ± 1.5 ^a
Striatum	468.8 ± 44.0 ^b	538.9 ± 40.5 ^c	
Cortex	65.8 ± 5.1	56.5 ± 5.0	
Adrenal glands	134.0 ± 29.3 ^b	156.7 ± 42.4 ^a	

Difference from respective controls significant at:

^a $p < 0.05$.

^b $p < 0.01$.

^c $p < 0.001$.

sensomotor cortex. The bores were filled with 3 M NaCl (recording electrode), 2 M acetylcholine, and 0.2 M norepinephrine bitartrate. The neuromediators were administered to the neurons with an electrophoretic current of 30 to 40 nA for 30 s, and 60-s recordings of the impulse activity before and after administration of the compounds were compared; the criterion of neuronal reaction was at least a 20% change in the pulsation rate. In total, 111 neurons have been thus registered in control and 48 in adapted animals. In the relative numbers of activated, unresponsive, and inhibited neurons upon acetylcholine and norepinephrine administration, the adapted animals differed markedly from the controls. The portion of unresponsive neurons in adapted animals increased 2 to 2.7-fold, while that of responsive neurons fell by 39 to 56% with the number of inhibited neurons declining to a greater extent. A more detailed study showed that the average reaction of neurons to acetylcholine and norepinephrine was much less pronounced in adapted animals. Thus with activated neurons the effect of norepinephrine was twofold in control, but only 34% in adapted animals; with inhibited neurons norepinephrine decreased their activity by 77% in control and by 34% in adapted animals. The responses to acetylcholine were also attenuated, albeit to a much smaller extent.

As shown in recent years, OPs can restrict the adrenergic regulation and other links of the stress reaction by several concrete mechanisms. First, at the presynaptic level the norepinephrine release from sympathetic terminals in the CNS and in the periphery can be suppressed by the action of OPs on the opiate receptors on the terminals.^{6,68,69} Second, OPs can also inhibit the adrenergic neurons themselves in the CNS and in the peripheral sympathetic ganglia,^{63,70} and block liberation of catecholamines from the adrenal glands and their action on the effector organs, which takes place through the postsynaptic opiate receptors.^{63,70} Third, besides restricting the activity and the effects of the adrenergic system, OPs can modulate the release of hormones. Beta-endorphin produced in the pituitary body has been shown to locally inhibit there the liberation of a stress hormone vasopressin.⁷¹ OPs inhibit the biosynthesis and liberation of corticosteroids, including glucocorticoids.⁷² On the other hand, OPs can activate the secretion of insulin,⁷³ usually depressed in stress, and secretion of thyrocalcitonin and somatotropin⁷⁴ participating in the adaptive effects of stress reaction (see Chapter 1).

In other words, OPs check the mobilizing effect of stress, preventing its transition to a detrimental one (Chapter 1). This was the basis for introducing OPs and their analogs to suppress the stress reaction and its injurious effects, in particular to prevent or attenuate the stress-induced and ischemic damage to the heart.

In stress caused in rats by 18-h hanging by the cervical fold, prior administration of a leu-enkephalin analog (0.1 mg/kg) substantially alleviated the complex of nonspecific stress damage (gastric ulceration, eosinopenia, and atrophy of the thymus) and checked the glucocorticoid elevation in blood.⁷⁵ Again, in stress caused by acute myocardial ischemia some leu-enkephalin analogs attenuated the stress-induced elevation of blood cortisol and prevented the rise in aldosterone,^{76,77} as well as attenuated twofold to threefold the stress increase in catecholamine excretion with urine.⁷⁸ Concurrently, OP administration before such stress prevented the stress decline in insulin secretion and the phenomenon of "stress diabetes".⁷⁸ Finally, prior administration of OPs efficiently prevented the rise in blood vasopressin in stress from acute myocardial ischemia or trauma.⁷⁶

It must be accentuated that restriction of the stress reaction with OPs is associated with prevention or alleviation of the stress damage and functional disturbances to the heart. Thus, we have shown that prior administration of beta-endorphin is effective against the stress-induced impairment of the contractile function of nonischemic myocardial regions in rats with acute experimental myocardial infarction.⁷⁹ Similarly, a leu-enkephalin analog dalargin substantially restricted myocardial damage caused by a 6-h EPS.⁸⁰ In this work, the damage was assessed by accumulation of labeled technetium pyrophosphate in the myocardium and by the creatine phosphokinase level in the serum, i.e., by the generally accepted criteria of cardiomyocyte membrane damage. Introduction of dalargin decreased more than by half the label accumulation at the peak of injury (12 h after stress) and reliably attenuated the stress elevation of the enzyme in blood. In the mechanism of protective action of OPs against cardiac stress injury, which is largely adrenergic in nature (see Chapter 1), an important place besides checking the catecholamine "discharge" is occupied by suppression of catecholamine action on the heart owing to adenylate cyclase inhibition through opiate receptors on the cardiac cells. Indeed, the adrenergic novodrin injury, displaying features of stress and ischemic damage and attended with release of cardiomyocyte markers into the blood, is associated with a rise in myocardial cAMP; prior administration of dalargin completely prevents this activation of adenylate cyclase,⁷⁶ i.e., virtually blocks the intracellular effects of adrenomimetics. Thus, *the protective effect of OPs against cardiac stress damage appears to involve their inhibitory influence on the centers of cardiac adrenergic regulation and also the influence on the heart proper where they inhibit norepinephrine release from sympathetic terminals and its action on the adenylate cyclase system.*

On this basis, we have recently for the first time used dalargin to prevent ischemic and reperfusion arrhythmias.⁸¹ Table 11 demonstrates that in intact animals pretreatment with dalargin decreases more than by half the overall duration of all types of arrhythmia both in ischemia and in reperfusion, including the grave forms such as ventricular fibrillation, the mean duration of which in reperfusion is four times less in dalargin-treated animals. It is quite essential that dalargin markedly diminishes the incidence of fibrillation and prevents death from fibrillation (Table 11). Thus, *in the whole organism dalargin exhibits a protective antiarrhythmic action in acute ischemia and reperfusion.* With isolated hearts, no such effect of this OP could be observed.

Attention should be paid to the fact that the opioidergic system is topologically and functionally linked not only with the stress-effecting, but also with other regulatory systems that now can be referred to as stress-limiting ones. In particular, it is most tightly connected with the serotonergic system, as shown by two lines of evidence. First, opiates and OPs markedly increase formation and liberation of serotonin from serotonergic neurons.⁸²⁻⁸⁴ Second, the analgetic action of OP is in large measure realized with the help of the sero-

TABLE 11
**Effect of Dalargin on Cardiac Rhythm Disorders in Acute
 Ischemia and Reperfusion in the Whole Organism**

Indices	Ischemia (10 min)		Reperfusion (10 min)	
	Control (n = 11)	Dalargin (n = 11)	Control (n = 10)	Dalargin (n = 10)
Extrasystole				
Number of animals	10	11	9	9
Total duration	259	243	66	92
Mean duration	24 ± 4	22 ± 7	7 ± 4	9 ± 4
Ventricular tachycardia				
Number of animals	11	9	10	9
Total duration	895	240	1364	467
Mean duration	81 ± 19	22 ± 8 ^a	136 ± 29	47 ± 13 ^a
Ventricular fibrillation				
Number of animals	8	2	5	2
Total duration	386	252	502	120
Mean duration	35 ± 17	23 ± 28	50 ± 36	12 ± 18 ^a
All arrhythmias				
Total duration	1540	735	1924	679
Mean duration	140 ± 22	67 ± 25	192 ± 43	68 ± 24 ^a
Deaths	1	1	5	0

^a Difference from respective controls significant at $p < 0.01$.

toninergic system and dependent on serotonin metabolism. In rats the antinociceptive activity of OP introduced into the cerebral ventricles is potentiated by serotonin and weakened by prior reserpination and depletion of the serotonin depot,⁸⁵ suppression of serotonin reuptake enhances,⁸⁵ while blocking the serotonergic receptors weakens the OP effect.⁸⁶ These data and the results of direct electrophysiological studies on the effect of OPs on interneuronal transmission at different levels on the CNS give grounds for supposing that OP can inhibit the interneuronal transmission of excitation, which in the spinal marrow takes place through activation of the serotonergic system by the opiate receptors of corresponding neurons.

In the words, *the emerging notion is that the stress-effecting system is coupled, through the opioidergic one or in a more direct way to the serotonergic system, whereby the latter acquires a stress-limiting function.*

Indeed, already in 15 to 30 min after the onset of immobilization stress in rats there is an elevation of serotonin in the hypothalamus and of its metabolite 5-hydroxyindole acetic acid in the hypothalamus, tonsil, hippocampus, and medulla oblongata.^{87,88} Serotonin, on the one hand, is involved in stimulating the hypothalamic production of the ACTH-releasing factor and ensuing secretion of ACTH and endorphins by the pituitary gland,⁸⁹ and on the other hand the OP-induced activation of the serotonergic system has been shown above to produce a stress-limiting effect itself. It is quite instructive that even when these notions were only emerging, Rabinowitz and Lown,⁹⁰ on the basis of the first data on the ability of serotonin to check the excitation of the brain adrenergic centers, undertook a successful attempt to achieve an antiarrhythmic effect by augmenting the serotonin concentration in the brain. This was done by combined administration of three substances: (1) the serotonin precursor tryptophan, (2) a decarboxylase inhibitor to interrupt tryptophan conversion to serotonin in all tissues except the brain, and (3) a monoamine oxidase inhibitor to prevent serotonin breakdown in the brain. The resulting elevation of the brain serotonin content produced a stable increase in the cardiac threshold for stimuli causing extrasystole.

We have recently undertaken a study⁹¹ of the effect of adaptation to stress on the serotonin content in the midbrain where in the region of the raphe there is a large congregation of

serotonergic neurons. The fluorimetric assay showed that the serotonin content increased by about 60%. Since such adaptation has a pronounced antiarrhythmic effect, at the next stage of our work we tried to prevent arrhythmias caused in conscious rats according to Lepran et al.⁴¹ with a synthetic serotonin analog 4-nitro-5-methoxytryptamine hydrochloride synthesized by Petrunin of the D. I. Mendeleev Chemical-Technical Institute, Moscow. Prior administration of this serotonin analog decreased threefold the total duration of all types of arrhythmia and fivefold the incidence of cardiac fibrillation in conscious acutely ischemic animals; mortality was completely abolished.

In the aggregate the above signifies that the opioidergic and linked serotonergic systems can restrict the stress reaction, and accordingly their mediators or synthetic analogs thereof have stress-limiting and specifically cardioprotective and antiarrhythmic effects. This is summarized in Figure 2.

Among other things, the proposed concept implies that genetic or acquired deficiency of the opioidergic system can augment the vulnerability of the organism to stress, whereas enhanced power of this system, conversely, can increase the organismic resistance to such influences. Indeed, in four lines of mice with differing innate density of opiate receptors in the brain this feature clearly correlated with the extent of analgesia in stress.⁹² Spontaneously hypertensive rats with enhanced activity of the sympathetic system⁹³ display a congenitally low OP content in tissues.⁹⁴ Furthermore, in myocardial infarction patients the severity of the stress component, pain syndrome, and enzymemia is the greater the lower is the beta-endorphin content in the blood.⁹⁵ This is the reason for adaptational or pharmacological activation of the opioidergic system, or "pharmacoprosthetics" with its mediators or their synthetic analogs.

IV. PARASYMPATHETIC SYSTEM, ITS CARDIOPROTECTIVE EFFECTS, AND ANTIARRHYTHMIC ACTION OF AN ACETYLCHOLINE ANALOG

Increasing vagal tone or vagal stimulation under certain conditions are known to raise the cardiac fibrillation threshold and even to stop the developing arrhythmias.⁹⁶ This is commonly thought to result from restriction of the excessive adrenergic influence on the heart in stress and ischemia.^{11,12} The adrenergic action is limited because acetylcholine acts as an agonist of muscarinic receptors to suppress norepinephrine release from sympathetic terminals^{97,98} and also restricts the response to norepinephrine at the receptor level.⁹⁸ Further, it should be borne in mind that in the heart the muscarinic receptors are coupled through guanine nucleotide-binding proteins with K⁺ efflux channels, and with the β-receptor-linked adenylate cyclase. Acetylcholine acting on the muscarinic receptors activates the K⁺ channels to enhance K⁺ efflux from the cell, but inhibits the adenylate cyclase to restrict the cAMP-dependent Ca²⁺ entry. This results in hyperpolarization and decreasing excitability of the cardiocyte membrane.^{99,100} Increasing concentrations of acetylcholine have also been shown to attenuate the potential-dependent entry of Na⁺ and Ca²⁺, which also favors hyperpolarization.^{100,101}

On the whole, the cardiac cholinergic apparatus restricts the excessive adrenergic stress effects on the heart, favors maintenance of a high enough RP of the myocardial cells, and thus acts as a stress-limiting antiarrhythmic factor.¹⁰¹

Obviously, through this mechanism, increasing tonicity of the cardiac parasympathetic regulation can play an important part in the protective, antiarrhythmic effect of adaptation to stress.

To test this suggestion, together with Kuznetsov we carried out experiments in which the mobility of rats was largely restrained by placing the animals in small boxes; this created a relatively mild immobilization stress which the animals experienced for 5 d and thus

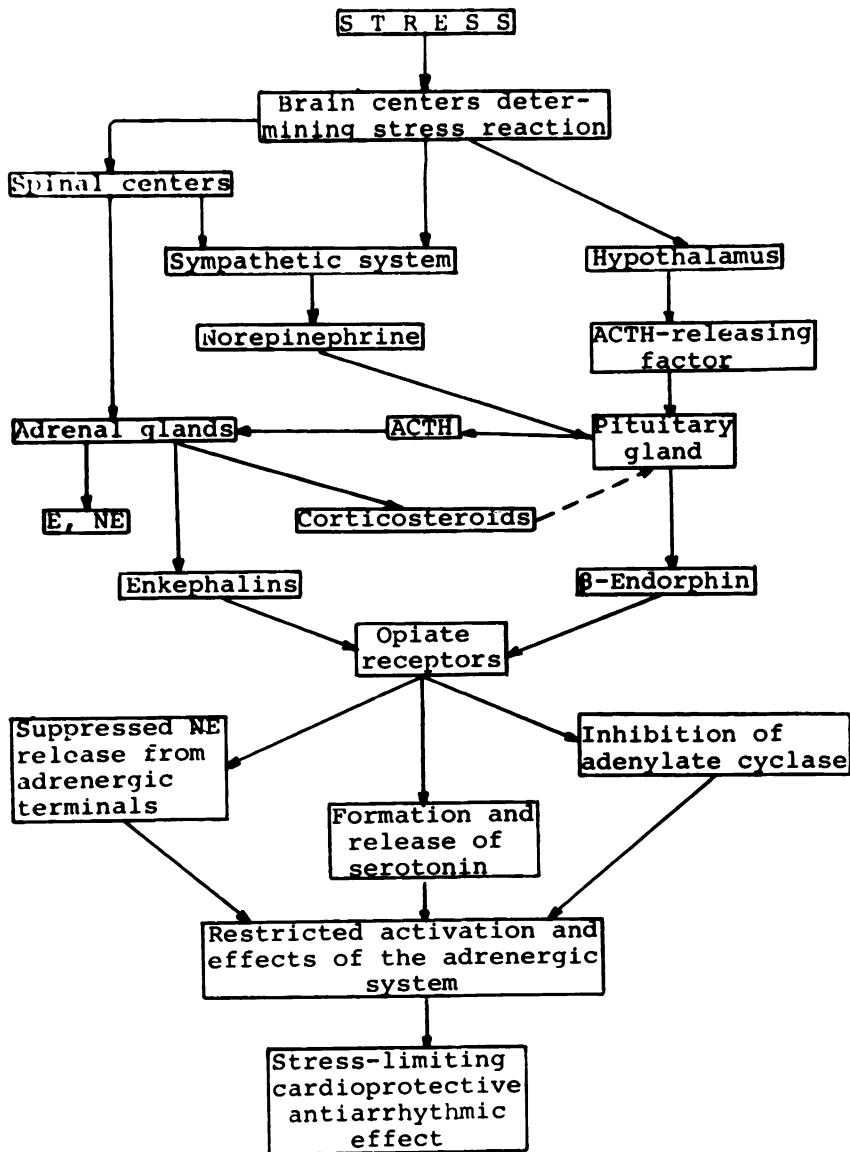


FIGURE 2. Coupling of the opioidergic system with the stress-effecting and the serotoninergic system; the stress-limiting cardioprotective effect of the opioidergic system. The broken arrow denotes the inhibitory influence of corticosteroids on ACTH and OP formation in the pituitary gland. For explanations see text.

adapted to this moderate stress. The results presented in Table 12 show that after 1 d of stress there was no appreciable change in the heart rate, whereas the ventricular fibrillation threshold decreased by half. By the fifth day bradycardia grew definite, while the fibrillation threshold was restored to normal. Administration of atropine at this time normalized the heart rate, but again decreased the fibrillation threshold. Thus, a 5-d adaptation to relatively mild stress resulted in increasing tonic negatively chronotropic vagal influence on the heart with concomitant restitution of the initially lowered cardiac fibrillation threshold.

Next, the animals were subjected to a 10-min ischemia followed by reperfusion as described earlier (see Chapter 3) to register the developing arrhythmias. Table 13 shows

TABLE 12
Rat Heart Rate and Fibrillation Threshold in Adaptation
to Continuous 5-d Stress and Blockade of the Cholinergic
Innervation

Series	Heart rate (min ⁻¹)	Fibrillation threshold (mA)
Control (n = 10)	441 ± 8	7.84 ± 0.24
1-d stress (n = 10)	413 ± 20	4.10 ± 0.57
5-d stress (n = 7)	354 ± 11	7.64 ± 0.58
5-d stress plus atropine (n = 8)	492 ± 13	5.07 ± 0.62
<i>P</i> ₁₋₂		<0.001
<i>P</i> ₁₋₃	<0.001	
<i>P</i> ₃₋₄	<0.001	<0.01

that adaptation decreased the overall duration of grave arrhythmias (ventricular tachycardia and fibrillation) more than sevenfold in ischemia and about threefold in reperfusion. In both cases this protective effect was abolished by atropine (10 mg/kg).

Thus, in the course of adaptation to continuous stress there is a gradual increase in the tonic vagal influence on the heart manifesting itself as bradycardia, normalization of the fibrillation threshold, and markedly enhanced resistance of the heart to the arrhythmogenic action of ischemia and reperfusion.

The gradually increasing tonic influence of the parasympathetic system on the heart in adaptation to continuous stress can serve as a natural stress-limiting antiarrhythmic factor. It is noteworthy that in this case resistance to such potent arrhythmogens as ischemia and reperfusion is enhanced not at the expense of heavy episodic exposures of the heart to negatively chronotropic influences, which can well be dangerous, but owing to a steadily enforced cholinergic regulation of the heart.

Basing on the principle of "imitating" the natural stress-limiting systems of the organism, we arrived at an idea that the new data on the role of parasympathetic regulation in antiarrhythmic mechanism of adaptation make topical a search for new cardioprotectors which would in full measure possess the adrenolytic and hyperpolarizing properties of acetylcholine, but would be free of its excessive negatively chronotropic effect, thus making optimal antiarrhythmics. It stands to reason that, first of all, attention should be paid to natural and synthetic compounds structurally close to acetylcholine. A family of this kind is formed by γ -butyrobetaine derivatives constantly produced in the brain and the heart.^{102,103}

An analogous compound, which was synthesized by Kalvins and co-workers in the Institute of Organic Synthesis, Latvian Academy of Sciences, and which we had an opportunity to test, is ethyl-3(2-ethyl-2,2-dimethylhydrazinium) propionate (EDIHYP).

Figure 3 shows the chemical structure of acetylcholine and EDIHYP; there is obvious similarity, yet certain differences in properties and activity could be expected, taking account of the fact that acetylcholine esterase does not hydrolyze γ -butyrobetaine esters.^{104,105}

Our studies with Abdikaliev were aimed at evaluating the antiarrhythmic properties of EDIHYP in clinically pertinent experimental models and elucidating the mechanism of its action.

The antiarrhythmic effects of EDIHYP were assessed in acute aschemia and reperfusion in anesthetized rats, in acute myocardial infarction, and in postinfarction cardiosclerosis. In all cases the drug was administered at 25 mg/kg *per os* 2 h before the tests. The main issues from the experiments were as follows:

1. In ischemia caused by a 10-min clamping on the left coronary artery, EDIHYP does not appreciably alter the number of extrasystoles, but decreases by half the mean

TABLE 13
Effect of Adaptation to Moderate Stress on Cardiac Rhythmn Disorders in Ischemia and Reperfusion with Intact and Blocked Cholinergic Innervation

Indices	Ischemia				Reperfusion			
	Control (n = 11)	Atropine (n = 11)	Adaptation (n = 8)	Adaptation + atropine (n = 9)	Control (n = 11)	Atropine (n = 11)	Adaptation (n = 8)	Adaptation + atropine (n = 9)
Extrasystole								
Number of animals	11	11	8	9	10	11	8	8
Total number of extrasystoles	324	421	178	737	219	305	70	204
Ventricular tachycardia								
Number of animals	6	6	2	5	10	10	6	7
Total duration	29	63	4	55	202	148	64	118
Mean duration	2.7 ± 2.5	5.7 ± 5.3	0.5 ± 0.7 ^a	6.1 ± 5.5 ^b	18.4 ± 5.2	13.4 ± 4.2	7.6 ± 4.4 ^a	13.1 ± 5.2
Ventricular fibrillation								
Number of animals	2	2	0	2	3	3	0	2
Total duration	11	13	0	7	45	78	0	18
Mean duration of grave arrhythmias	3.7 ± 4.0	6.9 ± 6.4	0.5 ± 0.7 ^a	6.9 ± 6.5 ^b	22.5 ± 9.3	20.4 ± 10.3	7.6 ± 4.4 ^a	15.1 ± 9.1

Difference with respective (a) "control" or (b) "adaptation" significant at $p < 0.05$.

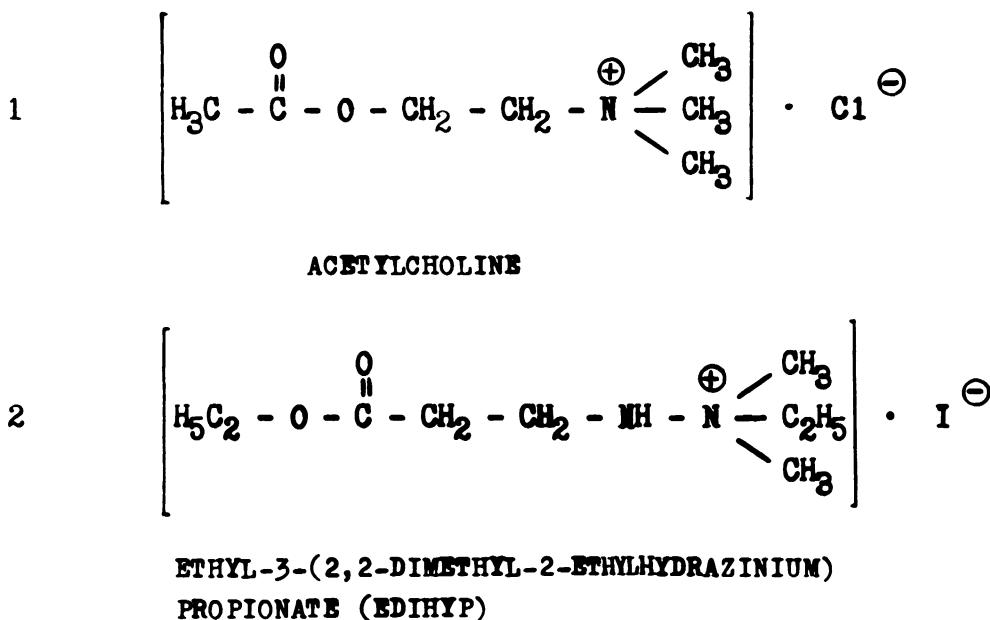


FIGURE 3. Structural formulas of (1) acetylcholine and (2) EDIHYP.

duration per animal of ventricular tachycardia, and diminishes 5-fold the incidence of fibrillation in acute ischemia (accordingly, the mean duration per animal of such arrhythmias falls 90-fold). In reperfusion the number of extrasystoles is decreased 2.5-fold, and mean durations of ventricular tachycardia and ventricular fibrillation decreased 5-fold and 3-fold, respectively. Thus, the antiarrhythmic effect of EDIHYP is greater in ischemic than in reperfusion arrhythmias.

2. In acute myocardial infarction and postinfarction cardiosclerosis, experimental therapy with EDIHYP abolishes the drop in ventricular fibrillation threshold commonly observed in these conditions, and decreases 7-fold to 8-fold the number of extrasystoles elicited in vagal bradycardia. Thus, *EDIHYP suppresses the ectopic activity of the heart in acute myocardial infarction and postinfarction cardiosclerosis.*

In studying the mechanism of this pronounced antiarrhythmic effect, we took account of the fact that the conditions of our model are rather close to the clinical ones, and on the other hand the most potent clinical antiarrhythmics are found among blockers of β -adrenoreceptors and of slow Ca^{2+} channels. This is explained by the fact that such compounds restrict or abolish the cardiotoxic action of high catecholamine and calcium concentrations, i.e., eliminate the two major pathogenetic links of arrhythmias. In this context we studied the adrenolytic and Ca-protective effects of EDIHYP. In all experiments with whole animals and isolated hearts we used atropine to check the extent to which the protective effect of EDIHYP as an acetylcholine analog is due to its binding with muscarinic receptors.

To assess the effect of EDIHYP on the cardiac damage by toxic epinephrine concentrations, four series of experiments were performed with isolated isotonically contracting hearts perfused according to Langendorf: (1) 15 min of perfusion with $5 \cdot 10^{-5} M$ epinephrine; (2) addition of $10^{-5} M$ EDIHYP before epinephrine; (3) $10^{-7} M$ atropine before epinephrine; and (4) EDIHYP, atropine, and epinephrine. In these experiments two issues have been established:

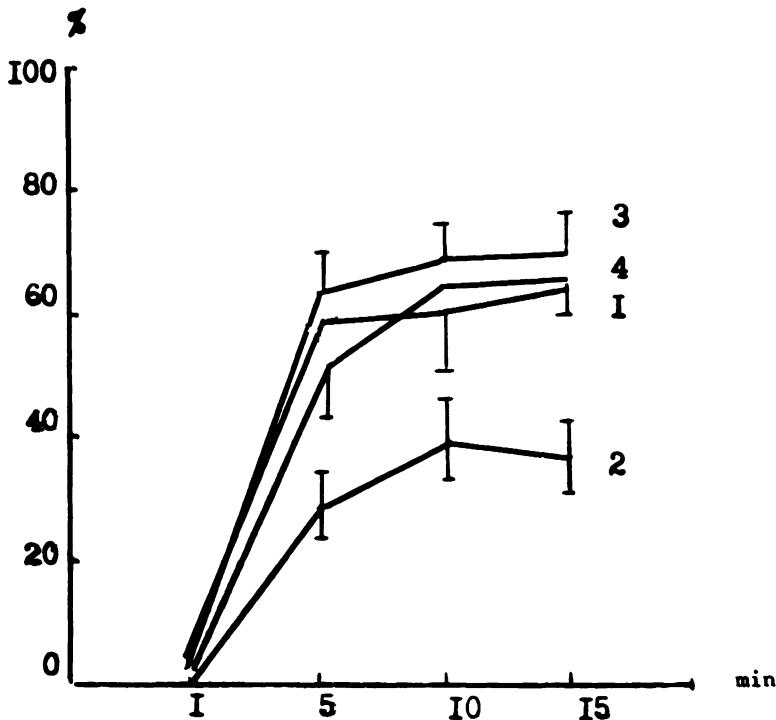


FIGURE 4. Effect of EDIHYP on the contracture caused with cardiotoxic doses of epinephrine. (1) Epinephrine; (2) EDIHYP plus epinephrine; (3) EDIHYP plus atropine plus epinephrine; (4) atropine plus epinephrine. The abscissa is time after administration, in minutes; the ordinate is extent of contracture, percent of the initial contraction amplitude.

1. Epinephrine at this concentration causes contracture of isolated hearts and decreases approximately threefold the main parameters of their contractile function: amplitude and velocity of contraction and velocity of relaxation. Figure 4 shows that EDIHYP reliably attenuates the adrenotoxic contracture, and by virtue of this checks the depression of the contraction amplitude. It can further be seen that atropine abolishes this anticontractural effect of EDIHYP which can therefore be supposed to take place through the muscarinic receptors, though, under these conditions it is not attended with a negatively chronotropic effect (no bradycardia is observable).
2. Moderate extrasystole (mean 77 extrasystoles per heart) develops by the tenth minute of exposure to epinephrine, which is reduced threefold ($p < 0.05$) with EDIHYP. Atropine by itself does not affect the extent of adrenotoxic arrhythmia, and in combination with EDIHYP does not change its antiarrhythmic effect. Hence, *the antiarrhythmic action of EDIHYP in adrenotoxic damage to the isolated heart does not involve the muscarinic receptors.*

In further studies, to assess the role of the antiadrenergic component in the protective effect of EDIHYP, we made use of a rather arbitrary model which is nevertheless accepted in modern pharmacology of arrhythmias, namely the arrhythmias evoked with intravenous administration of toxic doses (200 mg/kg) of calcium chloride; they are often regarded as the result of activation of adrenergic centers and enhanced discharge of catecholamines.¹⁰⁶ No matter how true is this opinion, the ability of some nontoxic substance to prevent at single administration the lethal arrhythmias caused by calcium chloride is obviously a reliable indication of its high antiarrhythmic activity.

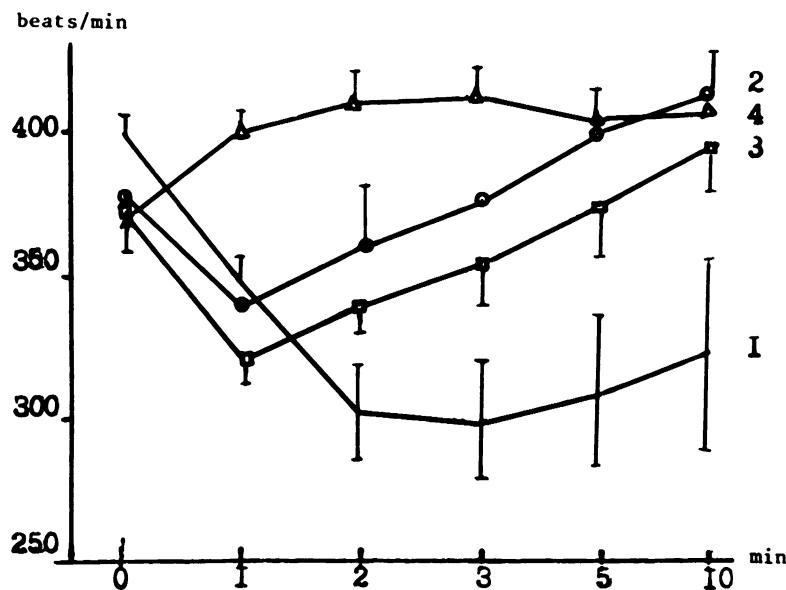


FIGURE 5. Effect of EDIHYP on the heart rate in CaCl_2 -induced arrhythmia. (1) CaCl_2 ; (2) EDIHYP plus CaCl_2 ; (3) atropine plus CaCl_2 ; (4) atropine plus EDIHYP plus CaCl_2 . The abscissa is time in minutes; the ordinate is heart rate in beats per minute.

In these experiments EDIHYP was administered at 5 mg/kg intravenously, 5 min before calcium chloride. The results are presented in Table 14 and Figure 5 and yield three main points.

1. EDIHYP administered before calcium chloride exhibits a pronounced antiarrhythmic effect. Indeed, in the control series 9 animals out of 15 died of fibrillation and ensuing cardiac arrest that occurred within 10 min following calcium chloride injection. The animals pretreated with EDIHYP developed no ventricular tachycardia or fibrillation and none of them died within the whole observation period. The mere comparison of mortality (60% in controls vs. none in protected animals) leaves no doubt about the powerful protection provided by EDIHYP against lethal CaCl_2 -induced arrhythmias.
2. The antiarrhythmic action of EDIHYP does not appear to involve a cholinergic component. Indeed, the "cholinergic" side effects — salivation and lacrimation — observed in the first minute after EDIHYP injection are completely abolished with a low dose of atropine (0.1 mg/kg, i.e., one tenth that used to block the muscarinic receptors) which does not at all attenuate the radical antiarrhythmic effect of EDIHYP.
3. The effect of EDIHYP and low doses of atropine in CaCl_2 arrhythmias is peculiarly synergistic. Atropine by itself at low doses does not appreciably change the number of extrasystoles or mortality, and even shortens by half the time from CaCl_2 injection to cardiac arrest (Table 14, $p < 0.001$), which is in line with the present day notion of the stabilizing action of the parasympathetic system on cardiac rhythm.¹⁰⁷ However, when introduced with EDIHYP, atropine not only does not interfere with its antiarrhythmic action, but even further diminishes the number of extrasystoles (Table 14). Moreover, Figure 5 shows that bradycardia observed in the first 10 min after CaCl_2 injection is only shortened, yet still manifest with either drug alone, but completely abolished with the combination of EDIHYP and low atropine.

TABLE 14
Effect of EDIHYP on the CaCl₂-Induced Arrhythmias

Indices	Control + CaCl ₂ (n = 15)	EDIHYP + CaCl ₂ (n = 15)	EDIHYP + atropine + CaCl ₂ (n = 15)	Atropine + CaCl ₂ (n = 15)
EExtrasystole				
Number of animals	13	6	2	9
Total number of extrasystoles	245	22	5	270
Ventricular tachycardia				
Number of animals	9	0	0	8
Total duration	236	0	0	135
Ventricular fibrillation				
Number of animals	10	0	0	6
Total duration	365	0	0	163
Mean time from CaCl ₂ to cardiac arrest	6.6 ± 2.0	6.6 ± 2.0	3.2 ± 0.4	
Deaths within 10 min from CaCl ₂	9	0	0	7

In the aggregate, the data obtained unequivocally testify to the pronounced protective action of EDIHYP against arrhythmias caused with toxic doses of calcium chloride. Thus, we are dealing with a promising antiarrhythmic.

Next, we assessed the ability of EDIHYP to check the contractual and arrhythmogenic action of high calcium concentrations on the isolated heart. Experiments were run with isotonically contracting hearts obtained from control animals and from those subjected to a 2-h immobilization stress. When the Ca^{2+} concentration in the perfusing solution was raised from 1.36 to 10 mM, pronounced contracture developed in the control hearts; it was still two to three times greater in the poststress hearts, more than half of which (6 of 11) went into fibrillation in 2 to 4 min of exposure to high calcium. The main result was that virtually no contracture or fibrillation was observed in the hearts of animals that received EDIHYP (25 mg/kg *per os* immediately before stress or 2 h before taking the hearts). *Hence, a single peroral administration of a relatively small nontoxic dose of this acetylcholine analog, without causing bradycardia, not only protects the heart from stress-induced impairment of its contractile function, but markedly enhances myocardial resistance to the cardiotoxic action of high calcium concentrations — calcium contracture and cardiac fibrillation. Since excess Ca^{2+} is a common central link of the pathogenesis of most various kinds of cardiac damage (viz. adrenergic, ischemic, reperfusion, hereditary cardiomyopathic), the discovered cardioprotective effect appears quite important.*

The pronounced antiarrhythmic and anticontractual effects of EDIHYP raised a question whether or not this acetylcholine analog can act on the muscarinic receptors *in vitro* when there is no competition from acetylcholine. A typical experiment presented in Figure 6 shows that introduction by a high EDIHYP concentration ($10^{-4} M$) into the perfusing solution of the isolated heart decreases the contraction rate by 100 beats per minute (cf. lanes a and b); addition of $10^{-7} M$ atropine almost completely abolishes this effect (lane c), although by itself atropine does not change the contraction rate (lane d). Thus, at a high concentration and without competition from acetylcholine existing in the whole organism, EDIHYP can act on the muscarinic receptors to cause bradycardia. The next question arising in this connection is whether or not this action is anyhow involved in the antiarrhythmic effect of EDIHYP or whether or not it can take place under complete blockade of the muscarinic receptors.

The answer comes from Table 15, showing the effect of $10^{-4} M$ EDIHYP, $10^{-7} M$ atropine, and their combination on ischemic and reperfusion arrhythmias produced in the isolated heart. We can see that either agent produces hardly any effect by itself, but together they prove highly protective in both conditions. The ischemic arrhythmias are known to be largely neurogenic in origin.^{11,12} and only weakly pronounced in the isolated heart; yet EDIHYP plus atropine decreases threefold to fivefold the number of hearts with extrasystole and the total number of extrasystoles as compared with other series. Their combined effect is still more striking in reperfusion; the mean duration of fibrillation is diminished by more than an order of magnitude, and all hearts keep contracting by the fifth minute of reperfusion when most of them have stopped in other series.

Summing up, though *in principle EDIHYP may produce a negative chronotropic effect via the muscarinic receptors, this has nothing to do with its antiarrhythmic action, which in the whole organism (in acute ischemia and reperfusion, myocardial infarction, or post-infarction cardiosclerosis, see above) is never attended with bradycardia or other cholinergic effects, and in the isolated heart is effectual just under blockade of the muscarinic receptors and absence of bradycardia.*

Of course, the exact mechanism of EDIHYP action is to be elucidated with the techniques of molecular pharmacology. At present it can only be supposed that this acetylcholine analog has a lower affinity for the muscarinic receptors than their natural agonist acetylcholine. Therefore, in the organism EDIHYP can usually add nothing to the tonic influence of the

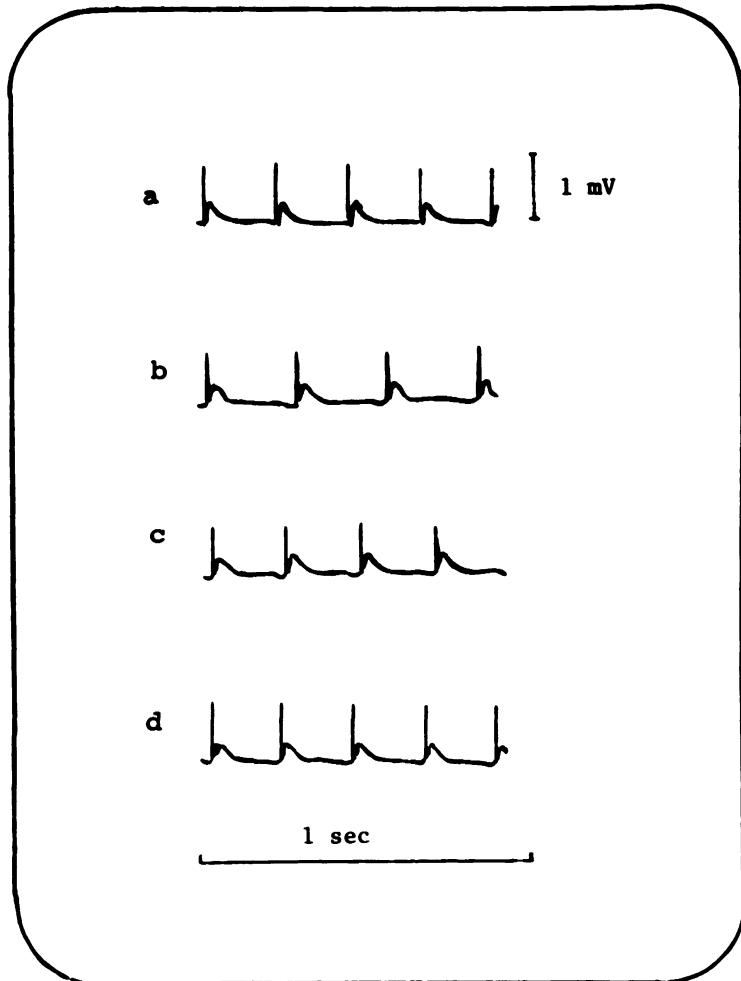


FIGURE 6. Effects of EDIHYP, atropine, and their combination on isolated heart contraction (electrograms). (a) Control; (b) 10^{-4} M EDIHYP; (c) 10^{-4} M EDIHYP plus 10^{-7} M atropine; (d) 10^{-7} M atropine.

parasympathetic cholinergic system on the heart and causes no bradycardia. It is obvious, on the other hand, that the powerful cardioprotective and specifically antiarrhythmic action of EDIHYP must involve some other regulatory mechanisms of the cell. To mention a couple of yet speculative possibilities, EDIHYP may act directly on the K^+ channels linked to the receptors via the regulatory G-protein,^{99,108} but unlike tetraethylammonium¹⁰⁰ does not block, but activates them, producing an acetylcholine-like hyperpolarization and enhancing thereby the myocardial resistance to arrhythmogenic factors. Again, EDIHYP may act like the pertussoid toxin¹⁰⁸ via the G-protein α -subunit to inhibit the adenylylate cyclase and to limit thereby the cAMP-dependent Ca^{2+} entry into the cells; this would hinder membrane depolarization in ischemia, reperfusion, or excess of catecholamines, contributing thus to the antiarrhythmic effect of EDIHYP.

This question, however, awaits further detailed and first of all biochemical studies with the use of labeled ligands and EDIHYP. In the context of this book, *it is noteworthy how the "principle of imitation" applied even to the most studied stress-limiting system such as the parasympathetic one can somewhat unexpectedly yield novel potent cardioprotective drugs.*

TABLE 15
Effects of EDIHYP, Atropine, and Their Combination on Rhythm Disturbances in Ischemia and Reperfusion of the Isolated Heart (in Each Series n = 10)

Indices	Ischemia				Reperfusion			
	Control		EDIHYP + atropine		Control		EDIHYP + atropine	
	Control	EDIHYP	EDIHYP + atropine	Atropine	Control	EDIHYP	EDIHYP + atropine	Atropine
Extrasystole								
Number of animals	7	8	2	3	8	8	10	9
Total number of extrasystoles	400	435	90	310	310	375	825	315
Ventricular tachycardia								
Number of animals	1	0	0	3	10	9	9	10
Total duration	28	0	0	26	535	412	133	325
Mean duration					54 ± 22	41 ± 12	13 ± 5	33 ± 8
Ventricular fibrillation								
Number of animals	0	0	0	0	8	7	5	8
Total duration					1516	978	82	1987
Mean duration					152 ± 29	98 ± 22	8 ± 8*	199 ± 28
Grave arrhythmias (VT + VF)								
Mean duration					205 ± 34	139 ± 25*	22 ± 13*	231 ± 34
Hearts working in 5 min of reperfusion					3	5	10	2

* Difference with control significant at $p < 0.01$.

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Chapter 5

LOCAL STRESS-LIMITING SYSTEMS AND THEIR CARDIOPROTECTIVE EFFECTS

I. INTRODUCTION

Operation of the stress-limiting systems exclusively by blocking or restricting the stress reaction at the central level can hardly be considered an optimal solution of the problem of modulating this reaction in the course of adaptation. Indeed, during adaptation to the changing set of environmental factors, in some working organs the strong effect of the stress hormones and mediators is absolutely indispensable, while in others it is quite needless. At the same time the stress reaction is known to be a generalized link of adaptation. Hence the most nearly perfect adaptation can in all probability be achieved owing to the participation of the local modulatory systems that either restrict or, conversely, potentiate the action of stress hormones and mediators in the organs and cells. Since the very concept of the stress-limiting systems is rather recent,^{1,2} the important question of the relations of the central and the local stress-limiting systems in general, and in the cardioprotective effect of adaptation specifically, has not been discussed heretofore.

As the reader will be able to see, the materials presented in Chapter 6 provide unequivocal evidence that all the protective effects of adaptation to repeated stress (enhanced resistance to toxic levels of catecholamines or calcium, etc.) are in full measure found in the isolated hearts of adapted animals. This issue, which will be the subject of special consideration, implies that besides the central neurohumoral regulatory systems an important part in the adaptational protection of the heart is played by local mechanisms forming in the course of adaptation in the organ. *A priori*, it can be envisaged that such local protection is provided by the antioxidant system, the adaptive changes in the calcium membrane transport, the adenosinergic and the prostaglandin systems, etc. Accordingly, we shall further deal with the data available to date on the changes in these systems in adaptation to stress and on the cardioprotective and first of all antiarrhythmic significance of such changes.

II. ANTIOXIDANT HEART PROTECTION FROM ISCHEMIC, REOXYGENATIVE, AND STRESS DAMAGES

Disturbances of the free-radical homeostasis, their role in cardiac injury, and methods of their correction constitute a rapidly developing trend of modern cardiology. Its origin can be traced back to two main sources from apparently remote fields of science. One is the fundamental studies of Bakh,⁴ Demenov,⁵ and Fridovich⁶ into the theory of free-radical oxidation. The other is the works in vitaminology^{7,8} demonstrating that the lack of α -tocopherol and other vitamins inhibiting free-radical oxidation may result in rapid injury to the cardiac muscle.

Further development of the notions of the role of free-radical reactions in cardiac pathology has gone through several stages.

Thus, when in 1969 superoxide dismutase (SOD) was discovered by McCord and Fridovich⁹ and proved to be highly active in the cardiac muscle, it became clear that cardiomyocytes had evolutionarily acquired a specific enzyme system for scavenging free radicals, and hence free-radical oxidation proceeds constantly in the myocardium.

In 1973, Hearse et al.³ described the oxygen paradox as cardiac muscle injury upon resumed supply of oxygen after a long enough period of anoxia. Owing to further studies by their laboratory and to the works of Guarnieri et al.¹⁰ on the antioxidant action of

TABLE 1
Activity of Antioxidant Enzymes and Content of LPO
Products in Heart Ventricles

Indices	Right ventricle (n = 10)	Left ventricle (n = 10)
Superoxide dismutase (arb. U/min·mg)	218 ± 21	333 ± 25 ^a
Catalase (nmol H ₂ O ₂ /min·mg)	256 ± 16	475 ± 25 ^a
Schiff bases (arb. fluorescence units)	1.64 ± 0.12	1.00 ± 0.15 ^a
Lipid hydroperoxides (OD _{234 nm} ^{1%})	1.49 ± 0.20	1.03 ± 0.13 ^a

* p < 0.05.

α -tocopherol and reduced glutathione, this important phenomenon got an explanation in terms of the free-radical oxidation theory. Finally, in 1976 Kogan et al.¹¹ obtained direct evidence for the involvement of free-radical oxidation in the pathogenesis of myocardial infarction, having demonstrated enhanced chemiluminescence in the ischemic zone and having attributed it to activation of free-radical processes. Simultaneously, our laboratory showed that activation of free-radical oxidation is a major element of the generalized stress damage to the organism (see Chapter 1). Emotional pain (EPS) and immobilization stress proved to cause accumulation of the lipid peroxidation (LPO) products in liver, lungs, heart, aorta, brain, and eye retina; antioxidants protected these organs from stress injuries. These data lay into the foundation of the concept of the role of LPO activation in the primary noncoronarogenic stress-induced damage to the heart.^{12,13} The mentioned studies reinforced the idea of the real significance of LPO activation in cardiac pathology, and now permit us to point out three basic mechanisms of LPO activation in the heart.

1. Primary excessive generation of active oxygen forms and lipid hydroperoxides in hyperbaric oxygenation, stress-induced excess of catecholamines, excess of variable-valency metals in hemolytic anemia, etc.
2. Primary decline in the power of the antioxidant systems in vitamin E deficiency, acatalasia, intoxication with enzyme poisons, etc.
3. Combination of primary LPO activation and deterioration of the antioxidant systems in hypoxia-reoxygenation, ischemia-reperfusion.

This concept predetermined the order of presentation in this section. We shall consecutively consider the mechanisms of LPO activation and the possibilities of the antioxidant protection of the heart in ischemia-reperfusion; LPO activation and antioxidant protection in cardiac stress injury; the role of LPO in arrhythmias and the experimental and clinical effects of antioxidants; and finally, some prospects for further studies.

In assessing the mechanism of enhancement of free-radical oxidation in ischemia and reperfusion, it should be borne in mind that in mitochondria, as electrons are transferred to cytochrome oxidase, under normal conditions there can occur a single-electron "side-drop" to dioxygen, giving rise to the active oxygen forms which react with the endogenous substrates in the cell structures, first and foremost with phospholipids. This free-radical lipid oxidation yields peroxide compounds and accordingly the whole process has been termed lipid peroxidation. Our data in Table 1 show that the LPO products are always present and hence the process is always operative in the normal ventricular myocardium. It can also be seen that the intensity of LPO is inversely correlated with the activity of the natural antioxidant agents.

LPO is enhanced in ischemia and reperfusion. Panel 1 in Figure 1 shows that normal four-electron reduction of oxygen on cytochrome oxidase proceeds in uninterrupted and

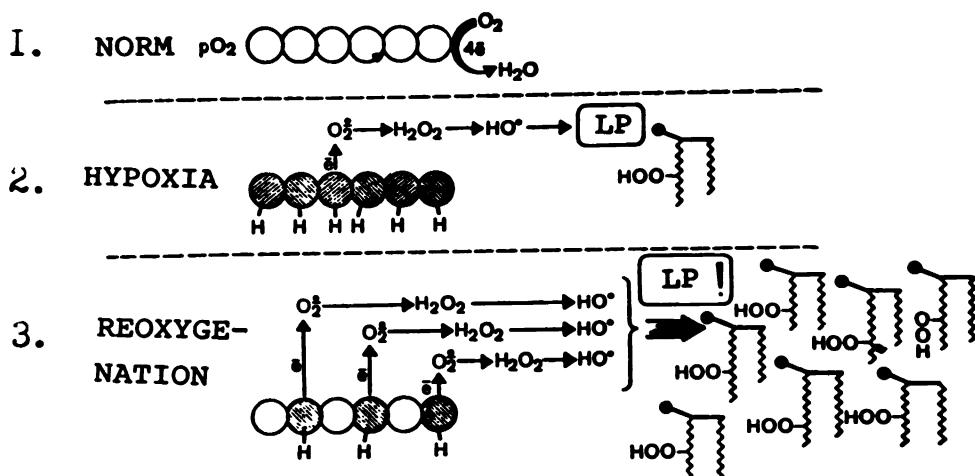


FIGURE 1. Activation of lipid peroxidation in hypoxia and reoxygenation. For explanations see text.

coordinated operation of the respiratory chain, i.e., at normal oxygen tension. Panel 2 shows that when the respiratory chain is blocked in its terminal link, as in ischemic hypoxia, the reduction of the preceding carriers causes the oxygen dissolved in the membrane lipid milieu to be reduced; however, this is not the four-electron reduction to water, but partial reduction producing active oxygen forms. Of course, with the lack of oxygen, accumulation of its active forms and LPO is only limited and slow. Panel 3 shows that much greater amounts of active oxygen and LPO products are formed when the reduced state of the respiratory-chain carriers (electron donors) combines with a high enough oxygen tension. This happens in reoxygenation following hypoxia and ischemia, i.e., after each coronary attack in recirculation through ischemic myocardial regions, and upon restoration of oxygen transport to the anoxic heart.

Initially, the active oxygen combines with unsaturated fatty acyl residues in phospholipids to produce unstable hydroperoxides. Their accumulation entails several rather important modifying effects studied *in vitro* by Arkhipenko et al.¹⁴

Figure 2A exemplifies, with the Ca ATPase of the sarcoplasmic reticulum (SR), one of the effects of this kind when hydroperoxides are accumulated and the amount of unsaturated lipids decreases in the lipid milieu of the integral proteins, which are the chief functional proteins of the membrane. Moderate LPO increases the mobility of the phospholipid chains, which as a rule is accompanied with enhanced catalytic activity of the enzymes. In pronounced LPO activation greater significance is acquired by the decreasing content of unsaturated phospholipids, which results in the integral proteins turning out to "freeze into" the more rigid milieu, with restricted conformational mobility of their polypeptide chains.

Figure 2B attempts to correlate these changes with the response of the isolated auricle to such an LPO inducer as hydrogen peroxide. It can be seen that initially H_2O_2 affects the atrial contractile function like catecholamines: the contraction amplitude increases and there is even a decrease in the tension at rest. Then the second stage develops, with pronounced bradycardia ending in cardiac arrest.

The second modifying effect is that of oxidized phospholipids accumulating as the result of LPO from ordered groups known as peroxide clusters. Figure 3 shows the process of their formation. Owing to the lateral diffusion of whole associations of oxidized phospholipids in each membrane layer, they come to be located opposite each other to form permeability channels allowing, in particular, passage of Ca^{2+} . Appearance of such clusters upon LPO activation caused by ischemia and especially reperfusion can be thought to play an important part in accumulation of excess calcium and its cardiotoxic effect.

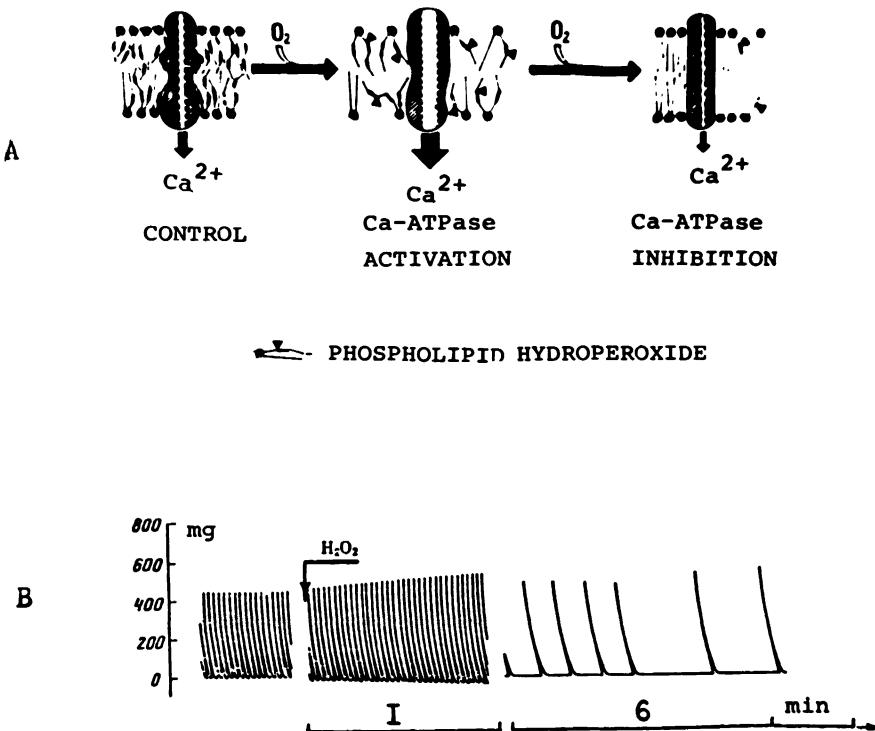


FIGURE 2. (A) Changes in the activity of the Ca ATPase of the SR as LPO proceeds in its lipid milieu, and (B) progressive disturbance to contraction of the isolated right auricle in exposure to H_2O_2 .

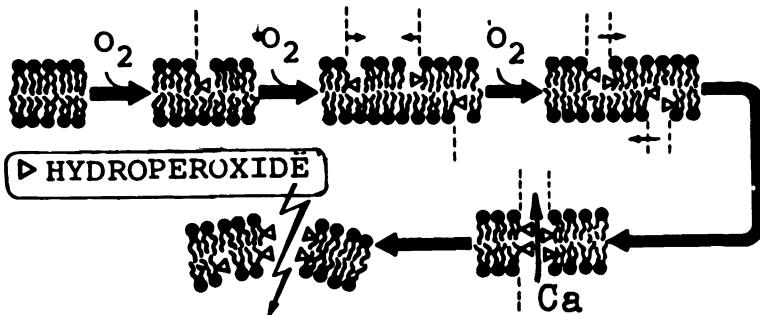


FIGURE 3. Formation of "peroxide clusters" and membrane fragmentation in LPO. The empty triangles denote peroxide groups. For explanations see text.

The above effects of LPO are essentially reversible. On the contrary, two other, namely protein intermolecular linking and inactivation of sulphydryl groups, appear irreversible.

Figure 4 shows one of these irreversible effects. The hydroperoxide produced in the reaction between phospholipid and activated molecular oxygen splits into a lysophospholipid-like compound with an abbreviated fatty chain and a short aliphatic dialdehyde which can interact with the amino groups of two protein molecules as a bifunctional linking agent.

Through these detrimental effects, LPO can cause more or less pronounced inactivation of the three important types of membrane proteins: cation pumps, ion channels, and signal-transmitting receptor systems. This impairs the electric stability and contractile function of

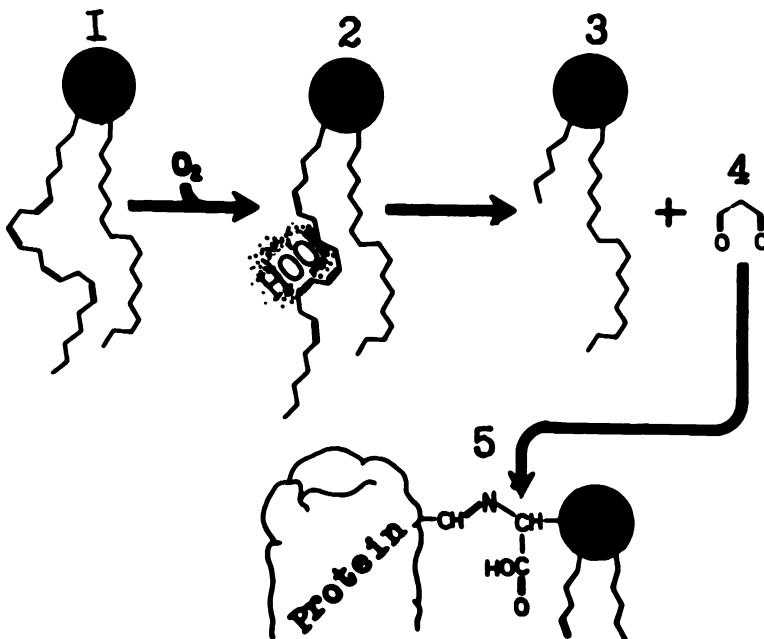


FIGURE 4. Formation of links and inactivation of membrane enzymic proteins in LPO.
For explanations see text.

TABLE 2
Main Mechanisms Activating Free-Radical Oxidation in the Myocardium in Ischemia and Reperfusion

1. Activation of free-radical lipid oxidation by electron carriers (see text)
2. Activation of free-radical lipid oxidation by the xanthine oxidase system^{15,16}
3. pH-dependent activation of free-radical lipid oxidation¹⁷
4. Generation of free radicals by leukocytes^{18,19}
5. Deterioration of the antioxidant system in cardiomyocytes²⁰⁻²²

the heart as a whole. Hence the problem of antioxidant protection acquires much clinical importance.

To evaluate the possibilities of antioxidant protection it is expedient to consider first the main mechanisms augmenting free-radical oxidation in the myocardium in ischemia and reperfusion; these are listed in Table 2.

The first one — formation of free radicals through partial reduction of oxygen by the respiratory chain — has already been discussed above. Other mechanisms of LPO activation are well known from recent works. Here it is reasonable to dwell on the last one listed, namely deterioration of the antioxidant system in the cardiomyocytes; indeed, were the myocardial antioxidant capacity unlimited, none of these factors could have caused LPO activation.

The scheme in Figure 5 shows that the cellular antioxidant mechanism operates as a cascade of specialized steps. Thus formation of the superoxide radical can be prevented by competing electron acceptors like vitamins E or K₃; conversion of the superoxide radical to hydrogen peroxide is blocked by anion-radical acceptors such as the sulfur-containing amino acids methionine and cysteine. Further, coordinated action of two enzymes — SOD and catalase — suppresses formation of hydroxyl radicals; since the latter readily interact with polyenic lipids, this enzyme system is central to regulation of LPO at the initiation stage.

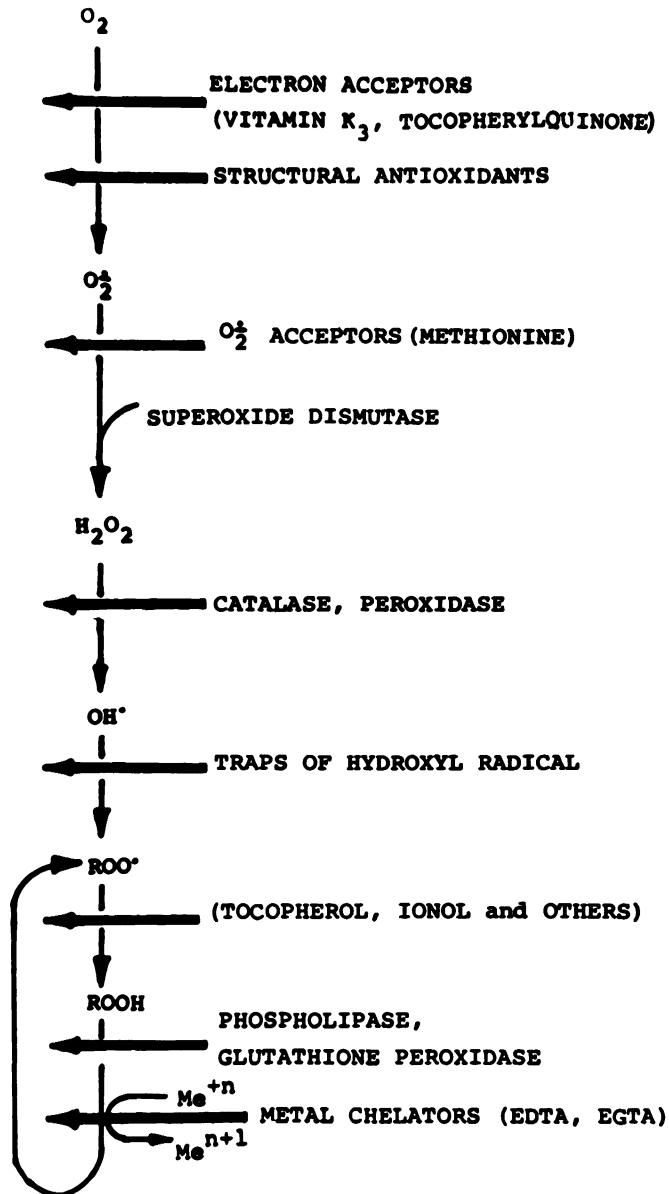


FIGURE 5. The antioxidant system of the cell.

The works of recent years in our laboratory have shown that the reliability of this antioxidant protection system is determined not only by its biological design, but also by its remarkable ability to increase its power upon repeated exposure of the organism to factors inducing LPO activation. This, in particular, is what happens in adaptation to repetitive stress.

Augmentation of the antioxidant system in the course of this process has recently been demonstrated with the use of a simple technique of inducing LPO by adding hydrogen peroxide or organic peroxides into the solution perfusing the contracting cardiac muscle.²³ When H_2O_2 was added to the oxygenated solution bathing the isolated auricles of control and stress-adapted rats, LPO was activated as manifest in accumulation of malonic dialdehyde

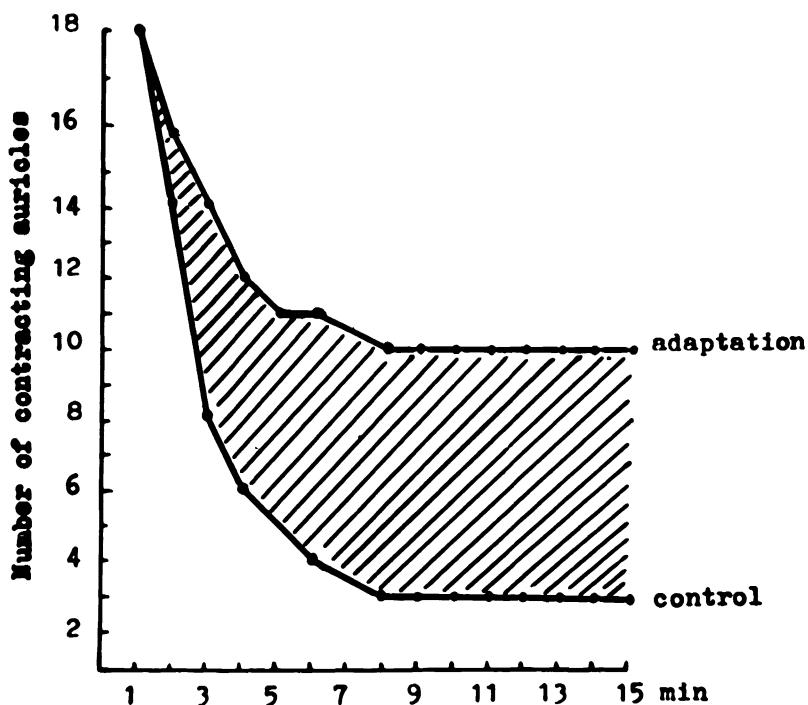


FIGURE 6. Effect of adaptation to short stress exposures on the resistance of isolated auricles to H_2O_2 -induced LPO. The abscissa is time after addition of H_2O_2 , min; the ordinate is the number of contracting auricles.

(MDA), and there were typical triphasic alterations in the atrial function ending in bradycardia and cardiac arrest.²⁴

Figure 6 compares the response of 18 control and 18 "adapted" auricles to an equal amount of H_2O_2 . One can see that within 10 min after adding the peroxide, 10 adapted, but only 3 control preparations continued to function, i.e., the auricles of adapted animals were appreciably more resistant to the LPO inducer. In accord with this, special experiments showed that upon LPO induction with the Fe/ascorbate system in the ventricular homogenates from adapted animals, MDA accumulated three times more slowly than in the control.²⁵ Thus, we observed actually increased resistance of the myocardium of adapted animals to factors inducing LPO.

The data in Table 3 shed some light on this phenomenon. As can be seen, after a single 6-h EPS the catalase activity in the myocardium declines by 20% while the activity of other antioxidant enzymes — SOD and glutathione peroxidase (GPO) — and the α -tocopherol content do not change appreciably. Upon adaptation to repeated stress catalase increases almost by half and SOD by 15%, while GPO and α -tocopherol remain the same. It should be recalled that catalase in the cells is essential to elimination of hydrogen peroxide which inhibits SOD; accordingly, their activities under optimal conditions change in synchrony,^{31,32} as observed in our case: in animals adapted to repeated stress and exposed to a single prolonged EPS the activities of antioxidant enzymes do not drop below control levels.

This activation of the antioxidant enzymes appears to be one of the factors of the cytoprotective action of adaptation to stress which, as we could have seen, does not at all decrease the ischemic zone in coronary artery ligation, but decreases the necrotic zone in infarction by over 40% (see Chapter 3) and checks the reperfusion arrhythmias (see above).

It is essential that not only adaptation to stress can augment the antioxidant capacity of

TABLE 3
Effects of Stress and Adaptation to Stress on the Activity of Antioxidant Enzymes
and α -Tocopherol Content

Indices	Control	Stress	Adaptation	Adaptation + stress	Assay (Ref.)
Catalase (nmol H ₂ O ₂ /min per g tissue)	1.82 ± 0.11 (100%)	1.47 ± 0.18 ^a (80.7%)	2.52 ± 0.27 ^a (138.5%)	1.96 ± 0.21 (107.8%)	26
Superoxide dismutase (arb. un./min per g tissue)	58.7 ± 2.1 (100%)	56.1 ± 2.1 (95.3%)	67.9 ± 2.5 ^a (115.7%)	64.0 ± 2.5 (109.3%)	27
Glutathione peroxidase (μ mol NADPH/min per g tissue)	19.2 ± 0.6 (100%)	18.7 ± 0.4 (95.8%)	19.0 ± 0.7 (98.9%)	19.4 ± 0.2 (101.2%)	28,29
α -Tocopherol (μ g/g tissue)	66.7 ± 2.1 (100%)	63.4 ± 4.3 (95.1%)	64.5 ± 2.9 (96.6%)	67.8 ± 2.6 (101.6%)	30

^a Difference with control significant at $p < 0.05$.

the organism and exhibit a cardioprotective effect. Adaptation of rats to swimming loads has been shown to increase the activity of antioxidant enzymes in skeletal muscle,^{2,33} which is accompanied by less pronounced activation of free-radical oxidation upon maximal physical loads than in untrained animals.³⁴ Similar changes are found in the myocardium of trained animals, with enhanced antioxidant activity correlating with the absence of cardiomyocyte membrane damage or enzymemia, which are consistently observed in untrained animals under maximal physical loads.³⁵ There are full grounds for believing that the enhanced activity of the antioxidant systems is one of the elements determining the widely known cardioprotective effect of training and specifically the attenuation of the IHD risk factors (a comprehensive review of this problem can be found in a recent monograph).²

Of course, this cytoprotective effect also involves other regulatory and first of all stress-limiting systems of the organism. In essence, the data on activation of the antioxidant systems in adaptation to stress or physical load mean that in using natural or synthetic antioxidants we, as in many other cases, follow the principle of mimicking the natural organic defense mechanisms.

It might seem that the efficient and adaptively reinforcing antioxidant system should safeguard the cell against excessive LPO and membrane damage. However, in point of fact this system is rapidly deteriorated in ischemia and reperfusion as shown by Gutkin and Petrovich.²⁰ According to their data depicted in Figure 7, 30 min after coronary artery ligation the leakage of SOD into the perfusate from the isolated heart increases to roughly the same extent as that of the marker enzyme lactate dehydrogenase. These authors observed no release of such important antioxidant enzymes as GPO and catalase, which was in line with the earlier data of Guarnieri et al.³⁶

However, further studies have shown that these two enzymes are inactivated in the ischemic myocardium. As is evident in Figure 8, GPO activity falls more than eightfold within 60 min (and most likely irreversibly) and SOD activity falls by half; these changes prove dramatic for the myocardial cells.

The scheme in Figure 9 depicts that familiar chain of events: the superoxide anion derived from several sources dismutates in the SOD reaction to a less dangerous compound, hydrogen peroxide, which is then disposed of by GPO and catalase. It is easily conceivable that the near-complete inactivation of GPO shown above would lead to accumulation of hydrogen peroxide. In the presence of substantial amounts of variable-valency metals, and first of all iron, this would inevitably result in immense activation of the Haber-Weiss reaction and intense generation of the rather toxic hydroxyl radical. This detrimental situation becomes self-sustaining, since the superoxide anion under conditions of acidosis can inhibit catalase, and hydrogen peroxide inhibits SOD. The combined action of the three active

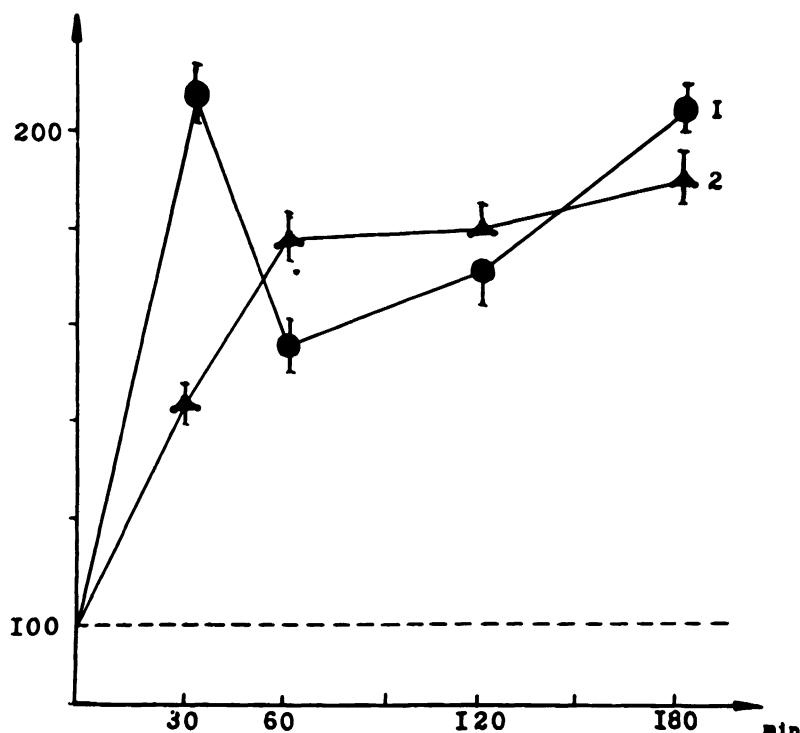


FIGURE 7. Leakage of lactate dehydrogenase (1) and superoxide dismutase (2) from the myocardium of the isolated heart into the perfusate upon coronary artery occlusion. The abscissa is time after occlusion in minutes; the ordinate is relative enzyme content in the perfusate, percent of that prior to occlusion.

oxygen forms results in profound damage to cell structures, increasing calcium concentration and its cardiotoxic effect, actuating or potentiating the whole pathogenetic chain of ischemic damage.

The phenomenon of ischemic breakdown of the myocardial antioxidant enzyme system at present raises no doubt, and in this case LPO is activated under conditions of a great lack of oxygen. This naturally raises the question, what is primary in ischemia: breakdown of the antioxidant system or enhanced free radical generation? Regardless of the answer, which is yet to be found, it can be stated that all the modern antioxidant protection in ischemia and reperfusion is based on compensating for the deficiency of the myocardial own system. Just this is the aim of administering α -tocopherol, ubiquinones, SOD, catalase, mannitol, glutathione, methionine, sodium selenite, or ionol, hydroxypyridines, and others.

The protective effects of these antioxidants observed by different authors are far from being similar. Table 4 presents the results from several laboratories that have tried to restrict the size of necrosis in ischemia and reperfusion using a number of antioxidants. The first three works listed demonstrate that 1 d after coronary artery occlusion the necrotic area is decreased by a nonspecific inhibitor of xanthine oxidase, allopurinol. However, in one of these works the effect was totally lacking when assessed after 2 d of ischemia.

Next is a group of important works where a short or rather long ischemia was followed by reperfusion of various duration. It turned out that in all cases antioxidants exhibit a protective effect even after 6 h of ischemia, but in subsequent reperfusion it is reliably observed only for a limited time, which usually does not reach 2 d. This has led the researchers

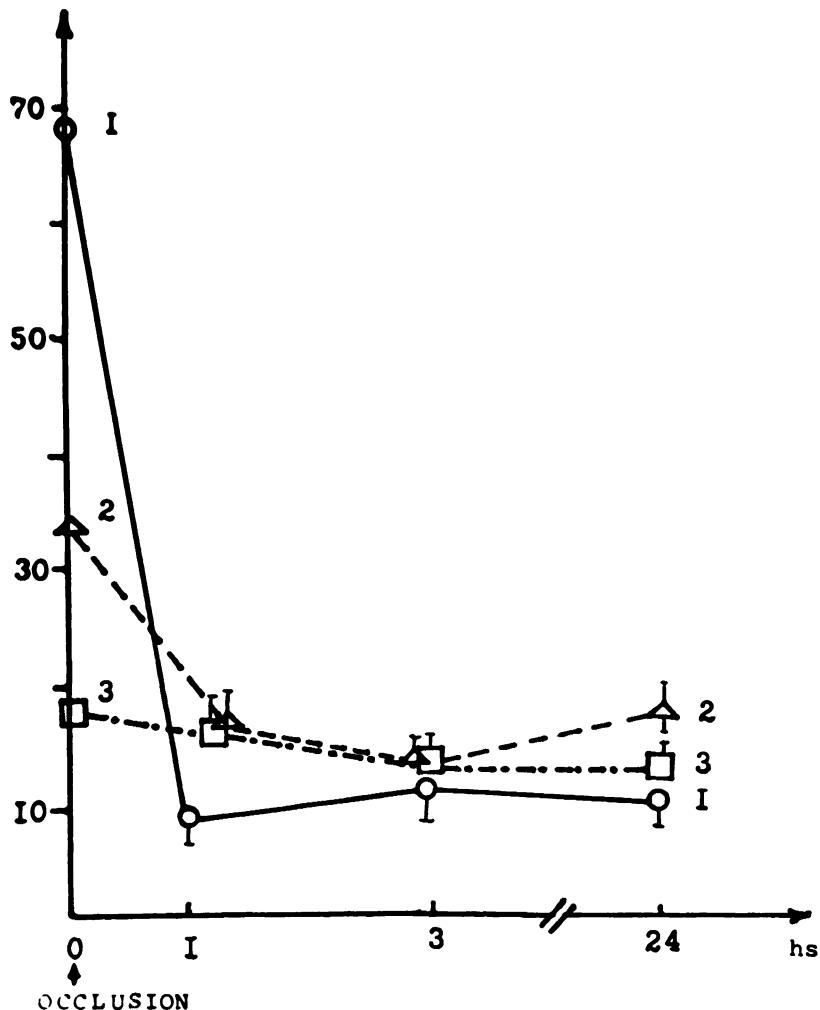


FIGURE 8. Changes in the activity of antioxidant enzymes in the zone of myocardial ischemia upon coronary occlusion. (1) Glutathione peroxidase, (2) superoxide dismutase, (3) catalase. The abscissa is time after occlusion, hr; the ordinate is enzyme activity: GPO, nmol/min; SOD, arb. units (inhibition of a O_2 -dependent reaction); catalase, μ mol/min.

to the common conclusion that the antioxidants only slow down the development of necrosis, but do not affect its final size.

We have studied the possibility of restricting the volume of necrotic tissue in experimental infarction with the synthetic antioxidant ionol (butylated hydroxytoluene) which, for half a century, has been used as a food additive.

In these studies⁴⁷ ionol was administered at 50 mg/kg in vegetable oil daily for 3 d before creating the infarction and for 2 d after. Myocardial infarction in rats was produced by left coronary artery ligation according to Selye.

The left part of Table 5 shows that 2 d after the infarction in the ischemic zone the content of LPO intermediates, dienic conjugates, is enhanced threefold and that of final LPO products, Schiff bases, fourfold. Administration of ionol has no effect whatsoever on these indices and hence on LPO activation. The right part shows that LPO activation in the ischemic zone is accompanied with a more than twofold decrease in GPO and catalase

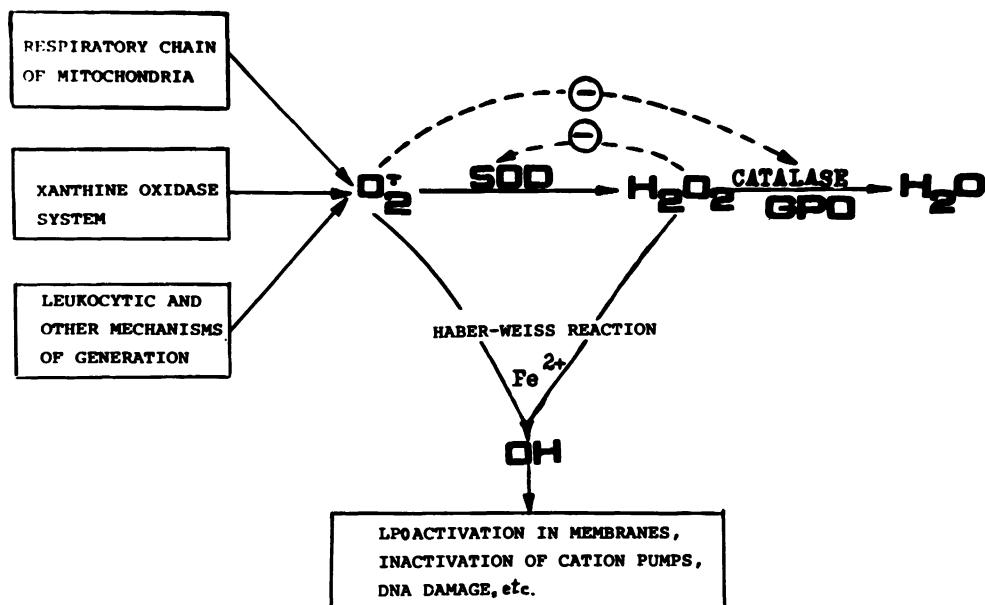


FIGURE 9. LPO activation and its detrimental effect in myocardial ischemia. For explanations see text.

TABLE 4
Effects of Antioxidant Agents on the Size of Ischemic Necroses

Agent	Object	Duration of		Necrotic size reduction (% of control)	Ref.
		Ischemia	Reperfusion		
Allopurinol	Dog	24 h	—	68	37
	Dog	24 h	—	63	38
	Dog	24 h	—	18	39
	Dog	48 h	—	0	39
	Dog	40 min	4 d	0	40
	Dog	60 min	4 h	50	16
SOD plus catalase	Rabbit	45 min	4 h	32	39
	Rabbit	45 min	3 d	0	39
	Dog	90 min	24 h	52	41
	Dog	6 h	—	45	42
	Dog	6 h	30—48 h	0	42
	Dog	90 min	6 h	45	43
Antiserum to neutrophils	Dog	90 min	24 h	37	44
	Dog	4 h	6 h	0	44
	Dog	90 min	2—3 d	30	45
Ibuprofen	Dog	3 h	3 d	0	46

activities and a somewhat lesser decrease in SOD. Neither of these changes is affected by ionol. This is the situation in the ischemic zone — in the left ventricle; it is different beyond, as shown in Table 6.

One can see that outside the ischemic region — in the right ventricle — LPO is also enhanced and the antioxidant enzymes are also partly inactivated. However, ionol practically abolishes these alterations. This difference may be attributed to greater activation of LPO and destruction of the local antioxidant system in the ischemic region, and inaccessibility of the ischemic zone for ionol.

At the same time it should be borne in mind that LPO activation outside the infarct

TABLE 5
LPO Products and Antioxidant Enzymes in the Ischemic Zone in Experimental Myocardial Infarction, and The Effect of Ionol

Indices	Control (n = 18)	Infarction (n = 18)	Ionol + infarction (n = 17)
Conjugated dienes (OD _{1 cm} ^{0.1%})	1.03 ± 0.08	2.91 ± 0.24 ^a	2.79 ± 0.24 ^a
Schiff bases (arb. units)	1.00 ± 0.14	4.30 ± 0.19 ^a	4.18 ± 0.14 ^a
Superoxide dismutase (un./min·mg)	29.6 ± 2.1	16.8 ± 1.08 ^a	18.2 ± 1.4 ^a
Glutathione peroxidase (nmol NADP/min·mg)	280 ± 11	115 ± 11 ^a	129 ± 17 ^a
Catalase (nmol H ₂ O ₂ /min·mg)	432 ± 30	198 ± 16 ^a	219 ± 23 ^a

* Statistically significant difference from control.

TABLE 6
LPO Products and Antioxidant Enzymes Outside the Ischemic Zone (Right Ventricle) in Experimental Myocardial Infarction, and the Effect of Ionol

Indices	Control (n = 18)	Infarction (n = 18)	Ionol + infarction (n = 17)
Conjugated dienes (OD _{1 cm} ^{0.1%})	1.36 ± 0.15	2.43 ± 0.29 ^a	1.53 ± 0.16
Schiff bases (arb. units)	1.37 ± 0.11	2.46 ± 0.22 ^a	1.41 ± 0.31
Superoxide dismutase (U/min·mg)	24.8 ± 0.9	21.4 ± 1.4	23.2 ± 1.6
Glutathione peroxidase (nmol NADP/min·mg)	241 ± 14	158 ± 14 ^a	222 ± 11
Catalase (nmol H ₂ O ₂ /min·mg)	356 ± 23	247 ± 17 ^a	298 ± 18 ^a

* Statistically significant difference from control.

TABLE 7
Effect of Ionol on the Volume of Necrotic Tissue as Assessed by Visual and Computer Morphometry

	Necrotic volume (% of cardiac muscle)	
	Visual point count	Computer image analysis
Myocardial infarction	38.5 ± 2.9 (n = 9)	48.1 ± 2.8 (n = 8)
Myocardial infarction plus ionol	28.8 ± 1.4	36.6 ± 1.7

Note: p < 0.05.

results not from ischemia or reperfusion, but from the stress adrenergic attack which attends any myocardial infarction; it is consistently observed in EPS and prevented with β-blockers as well as with various antioxidants. In this connection we have estimated the influence of ionol on the size of necrosis in experimental infarction.⁴⁸ Table 7 shows the volume of necrotic tissue (percent of left ventricular myocardium) determined in sections stained for succinate dehydrogenase: the necrotic portion reaching 48% in unprotected animals is decreased by one fourth in ionol-treated ones.

This positive result might reasonably be explained by the cytoprotective effect of ionol in the periphery of the ischemic zone to which it had some access; however, the very fact of restriction of the necrotic area remains debatable.

It is known that ischemia and reperfusion are attended with catecholamine- and calcium-induced activation of phospholipases, detergent action of lysophospholipids and acylcarni-

tine, lysosome labilization, and release of lysosomal enzymes. Simultaneously, the lack of energy incapacitates the cation pumps, which in its turn leads to calcium myofibrillar contracture, cardiomyocyte swelling, etc. Thus, LPO activation is just one of the links of the pathogenetic chain of ischemic damage (see Chapter 2). This particularly appears to account for the limited extent and instability of the antinecrotic action of antioxidants in myocardial infarction. Notwithstanding, this gives no grounds for being pessimistic in considering the problem of heart protection with antioxidants. The matter is that LPO is activated in non-ischemic regions of the heart under the influence of ischemic stress, throughout the heart in EPS as well as in reoxygenation following transient ischemia; it plays an important part in the pathogenesis of arrhythmias and in cardiac injury in hemolytic anemia;^{12,49} it is also consistently elicited in response to large physical loads and decreases physical endurance in animals and humans.⁵⁰ In all these cases there is reversible but potentially dangerous injury to the cell membrane structures. Hence there is a need for efficient and differentiated antioxidant protection, and the problem is no less important than that of attenuating the myocardial necrosis in infarction. Accordingly, we shall further consider (with ionol and some other antioxidants) the possibilities of antioxidant protection in myocardial infarction, cardiac stress damage, arrhythmias, and physical loads.

Figure 10 presents the dynamics of left-ventricular developed pressure and contraction velocity at relative rest and under maximal load imposed by clamping the aorta for 25 s. One can see that both indices are substantially decreased in animals with experimental myocardial infarction, the depression being greatest by the 25th second of clamping as the remaining myocardial regions grow tired. Prior administration of ionol prevents such depression to a large extent.⁵¹ This beneficial effect can in great measure be attributed to ionol protecting the nonischemic myocardial regions against the stress damage attending infarction. Indeed, Figure 11 shows that the marked decrease in the right atrial contraction force is largely abolished with the antioxidant.⁵²

It is quite plausible that similar protection took place in the nonischemic regions of the ventricle, providing for its higher contractile function as shown in Figure 10.

Notably, administration of the antioxidant restricts not only the depression of contractile function, but also the disturbances of cardiac electric stability in infarction.⁵³

Table 8 demonstrates the influence of prior administration of ionol on the fibrillation threshold and the ectopic activity of the heart, which are affected in myocardial infarction. The fibrillation threshold is more than threefold lower 2 d after infarction, and in vagally induced bradycardia numerous extrasystoles are observed. Ionol given for 3 d before and after infarction completely prevents the drop in the fibrillation threshold and diminishes six times the number of vagally elicited ventricular extrasystoles.

These data do not at all mean that ionol specifically should be used in human myocardial infarction, but point to the basic possibility of substantial antioxidant protection against disturbances of cardiac electric stability and contractile function in infarction. We are inclined to regard such protection as the result of restriction of the detrimental action of stress inevitably attending myocardial infarction.

The main mechanism by which stress damages the myocardium, and in particular the nonischemic heart regions in infarction, has been considered in detail in Chapter 1; the primary stress-induced noncoronarogenic damage is an independent phenomenon not linked to ischemia. Indeed, the works of Verrier and other researchers (see Chapter 1) have proved that in the heaviest behavioral stresses conscious animals display not constriction, but, contrariwise, adrenergic dilatation of the coronary vessels and enhancement of the coronary blood flow which, however, is accompanied by a significant drop in the cardiac fibrillation threshold. At the same time, our laboratory has shown that severe exogenous stress gives rise to a peculiar complex of damage which fits well into the concept of the "primary stress heart". In this case we are dealing with the β -adrenergic damage that closely resembles the

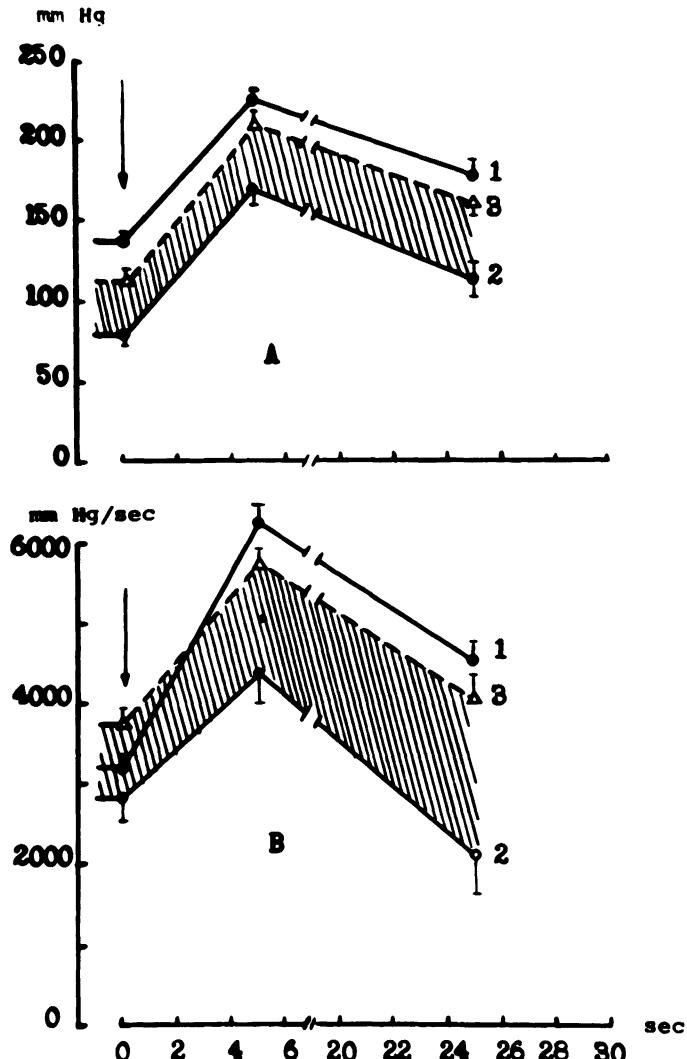


FIGURE 10. Attenuation of the depression of left-ventricular contractility in experimental myocardial infarction with the antioxidant ionol. (A) Developed pressure and (B) pressure buildup rate at relative physiological rest and under maximal cardiac load imposed by complete clamping of the aorta. (1) control, (2) infarction, (3) ionol plus infarction. The shaded zone reflects the protective effect of ionol. The arrow denotes the moment of aortic clamping.

isoproterenol-induced lesions first described by Rona et al.⁵⁴ in 1959. Nowadays there is much to make one believe that this complex is not just a lab finding, but an important pathophysiological and clinical syndrome — the primary stress damage to the heart extensively considered in Chapters 1 and 2.

In the present context, the main point of interest is that the chief constituents of cardiac primary stress damage are in great measure induced by LPO activation and can therefore be prevented with timely administration of antioxidants.

A 6-h EPS has been shown to cause accumulation of dienic conjugates and Schiff bases in the myocardium, which can be attenuated by pretreatment with ionol (Chapter 1).

Figure 12A shows that upon EPS the activity of the sarcolemmal Na,K ATPase is

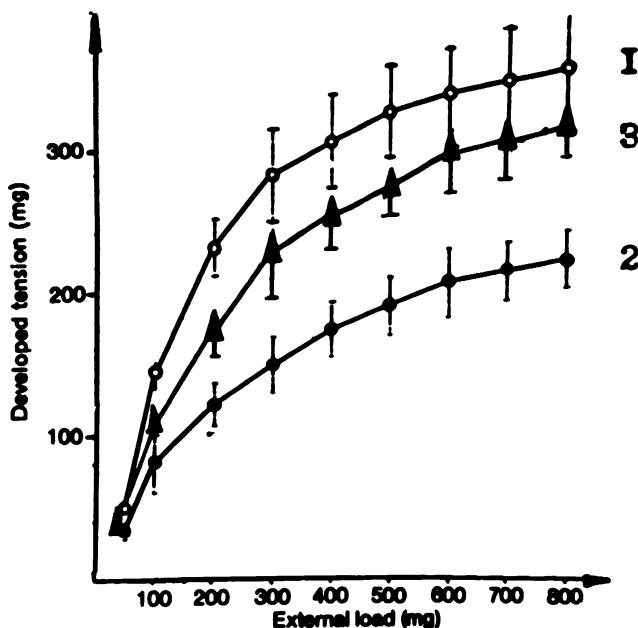


FIGURE 11. Effect of prior administration of ionol on the depression of right-atrial developed tension in experimental left-ventricular infarction. The abscissa is the stretching load, milligrams; the ordinate is developed tension, milligrams. (1) Control, (2) infarction, (3) ionol plus infarction.

TABLE 8
Effect of Ionol on Cardiac Electric Stability in Myocardial Infarction

Series	Heart rate (beats/min)	Fibrillation threshold (mA)	Number of extrasystoles in vagal bradycardia per group
Control (n = 10)	404 ± 13	7.0 ± 0.4	0
Myocardial infarction (n = 10)	394 ± 17	2.1 ± 0.2*	167
Ionol (n = 10)	409 ± 12	6.5 ± 0.3	0
Ionol plus infarction (n = 10)	386 ± 16	7.3 ± 0.3	27

* p < 0.001.

lowered and its thermal inactivation accelerated. Ionol (Figure 12B) prevents this: the shaded zone reflecting the effect of stress almost completely disappears.

As can be seen in Figure 13, the uptake of Ca^{2+} is impaired in SR vesicles isolated from the stress heart; the shaded zone here reflects the protective effect of the antioxidant.

The antioxidant protection against stress can prevent all disturbances to cardiac contractile function, metabolism, and, what is most important, to its electric stability as demonstrated in Figure 14, where prior administration of ionol virtually abolishes the stress-induced decline in the fibrillation threshold (A) and aggravation of vagal bradycardia (B).

These facts provide unequivocal evidence for the feasibility of preventing stress damage with antioxidants; they are important because pronounced LPO activation has now been proved for humans under stress (see Chapter 1) and because we could see that stress damages persist even after a single exposure to stress.

In real life, man, like animals, goes through countless stressful episodes, whose consequences may add up to evolve into noncoronarogenic, nonischemic cardiosclerosis that

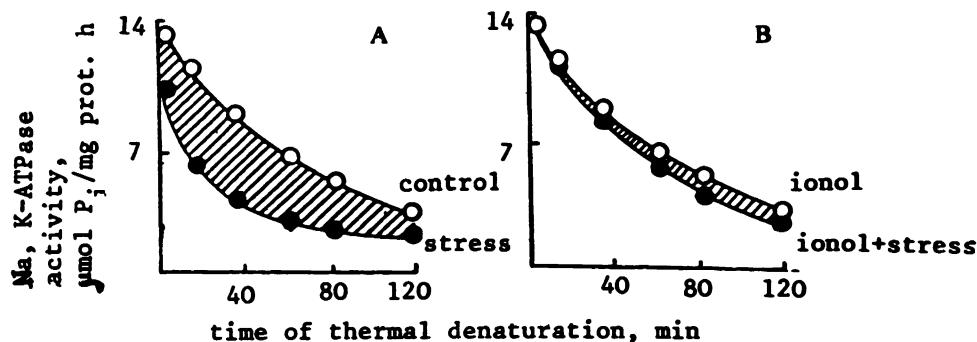


FIGURE 12. Accelerated thermoninactivation of the Na₊,K₋ATPase in emotional pain stress (A) and prevention of this with ionol (B).

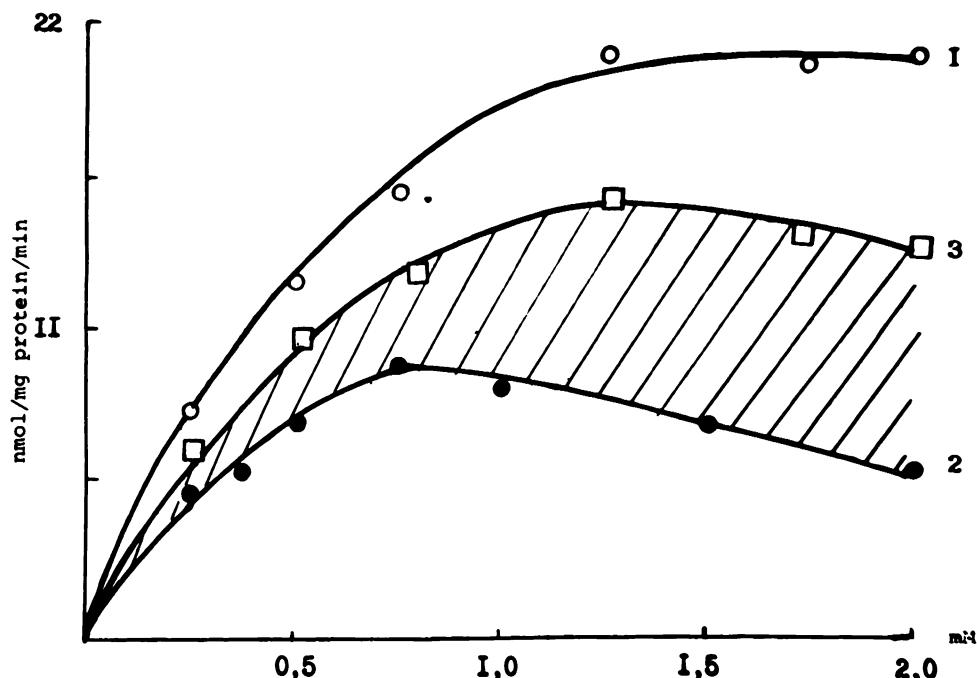


FIGURE 13. Attenuation of the stress-induced impairment of Ca²⁺ uptake by cardiac SR vesicles with ionol. The abscissa is Ca²⁺ concentration in the medium, mM; the ordinate is the rate of Ca²⁺ uptake, nmol/min per milligram protein. (1) Control, (2) stress, (3) ionol plus stress.

is, in our opinion, involved in heart diseases. This first of all applies to a large number of patients who have no ischemic disease, but suffer from stubborn, so-called idiopathic arrhythmias and blocks in various divisions of the conduction system, and sometimes from idiopathic cardiopathies leading to heart failure in patients without previous circulatory disorders. In many cases the idiopathic arrhythmias and cardiopathies are probably of stress origin. Therefore, a high content of natural antioxidants in food and special antioxidant protection in stress may attenuate the number of such often serious patients.

Of special significance is the antiarrhythmic action of antioxidants, since the combination of ischemia and stress is the main cause of grave arrhythmias, fibrillation, cardiac arrest, and sudden cardiac death. This situation can be most unambiguously reproduced with acute

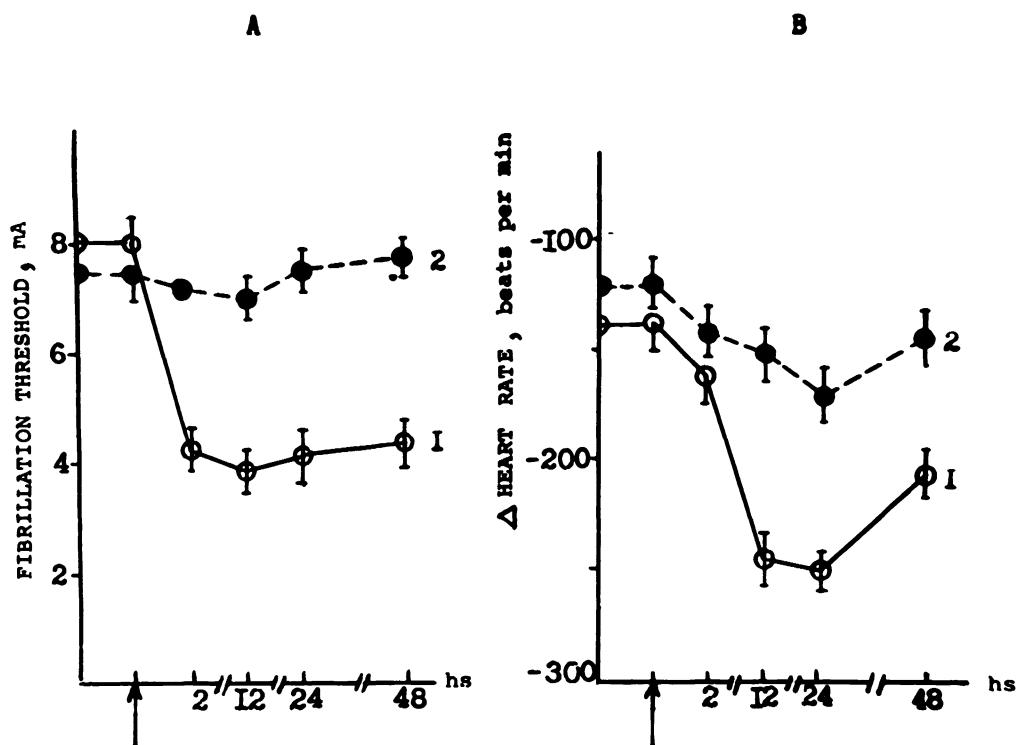


FIGURE 14. Effect of prior administration of ionol on (A) cardiac electric stability and (B) extent of vagal bradycardia in rats that have undergone stress. (1) Untreated animals; (2) ionol-treated animals. Arrows indicate stress. The abscissa is time after stress in hours; the ordinates are (A) fibrillation threshold, milliamperes; (B) drop in the heart rate upon vagal stimulation, beats per minute.

coronary artery occlusion in conscious animals; therefore, we thought it expedient to assess the potentialities of antioxidant protection in just these conditions.

The ECGs in Figure 15 reflect the results obtained with left coronary artery occlusion in conscious rats according to Lepran et al.⁵⁵ Ionol, though not abolishing the pronounced ischemic ECG alterations typical of coronary occlusion, nevertheless prevents ventricular tachycardia and fibrillation. Moreover, mortality within 20 min of occlusion was close to 60% (12/21) in the control unprotected group and four times less (3/21) in the ionol-pretreated group.

It is noteworthy that the antiarrhythmic action of antioxidants, unlike their antinecrotic action, has been unequivocally proved in many authoritative laboratories worldwide. Table 9 reviews the antiarrhythmic effects of a broad spectrum of antioxidants in reperfusion (i.e., the most severe) arrhythmias. One can see that antioxidants with quite different mechanisms of action — from xanthine oxidase inhibitors to iron-binding agents — are capable of reducing arrhythmias.

There is also a considerable body of testimony to the antiarrhythmic action of antioxidants in ischemia and adrenergic attack (see review).⁶³ All this means that antioxidants ought to have an important enough place in the panoply of modern antiarrhythmics.

By and large, collating the marked antiarrhythmic, antistress action of many antioxidants and their debatable effectiveness in restricting the size of ischemic necrosis, one cannot help concluding that the prospects for their use lie mostly in protection against stress and cardiac arrhythmias rather than against irreversible ischemia and necrosis.

Since neurogenic mechanisms are pathogenetically important in arrhythmias, the use of

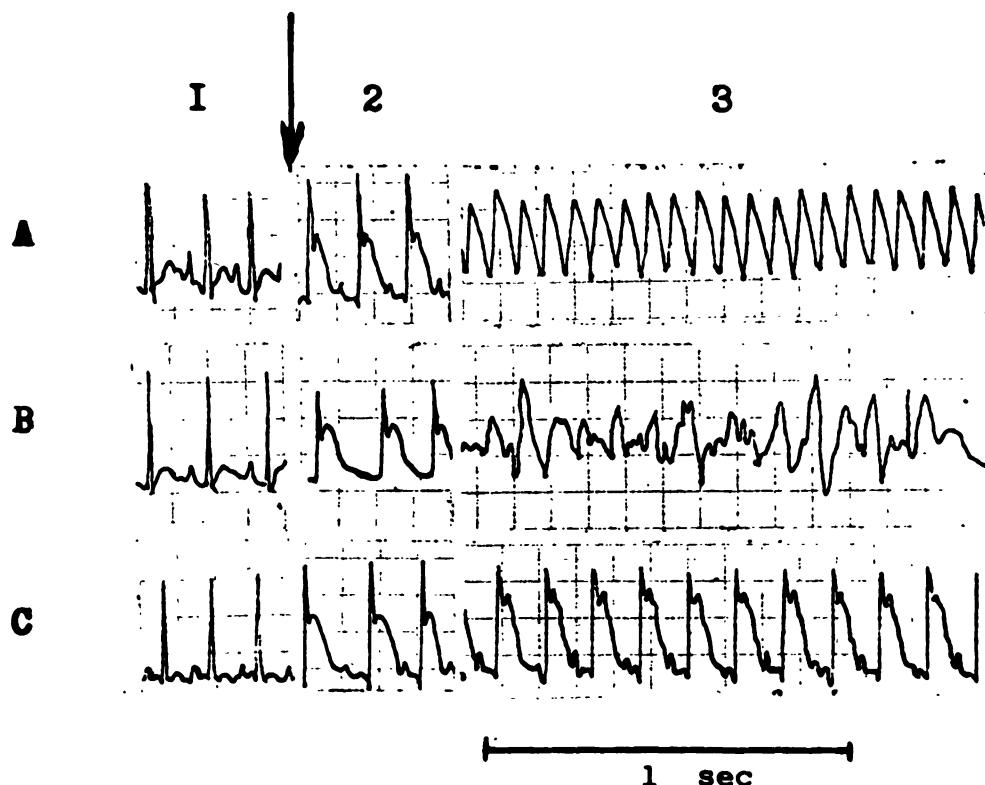


FIGURE 15. Effect of prior administration of ionol on cardiac rhythm disorders elicited by acute coronary occlusion in conscious animals. ECG (I indirect lead): (1) before, (2) 30 s, and (3) 5 min after occlusion. The arrow indicates the moment of occlusion. (A,B) Control, (C) ionol. Explanations in text.

TABLE 9
Effects of Antioxidants on the Incidence of Reperfusion Arrhythmias

Agent	Object	Conditions	Effect*	Ref.
Allopurinol	Rat	Anesthesia	D	56
	Dog	Anesthesia	N	57
SOD	Rat	Isolated heart	D	58,59
	Rat	Anesthesia	N	60
Catalase	Rat	Isolated heart	D	59
	Rat	Anesthesia	N	60
Glutathione	Rat	Isolated heart	D	58,59
Mannitol	Rat	Isolated heart	D	58,59
Vitamin E	Rat	Anesthesia	D	60
Vitamin C	Rat	Isolated heart	D	58
Coenzyme Q ₁₀	Dog	Anesthesia	D	61
Folic acid	Rat	Anesthesia	D	62
Amflutisol	Rat	Anesthesia	D	62
Desferrioxamine	Rat	Isolated heart	D	59
L-Methionine	Rat	Isolated heart	D	59

* D, decrease; N, no effect.

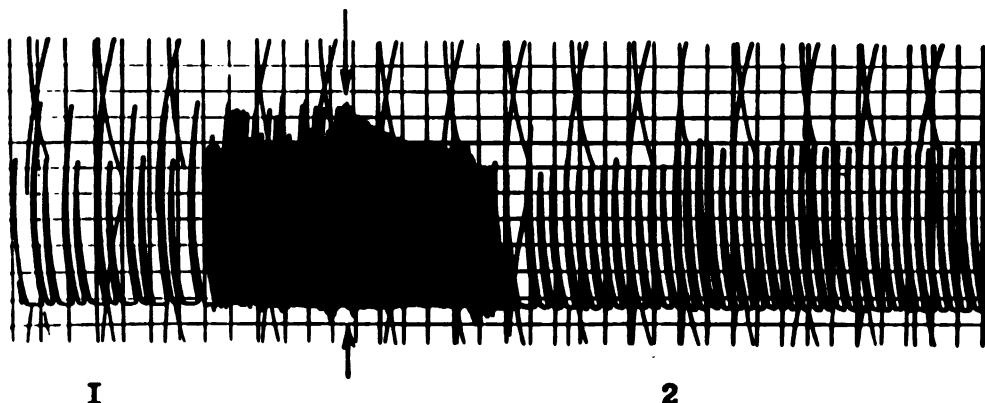


FIGURE 16. A hydroxypyridine antioxidant abolishes spontaneous arrhythmia in the isolated auricle. (1) Spontaneous bigeminy; (2) normalized rhythm. The arrow denotes addition of the antioxidant. Explanations in text.

TABLE 10
Effect of Ionol on Neuronal Uptake of ^3H -Norepinephrine and Synthesis of ^3H -Catecholamines (10^3 cpm/g Tissue) and on the Catecholamine Content ($\mu\text{g/g}$) in Rat Adrenal Glands in Stress

Indices	Series (n = 12 each)			
	Control	Stress	Ionol	Ionol + stress
Neuronal uptake of ^3H -norepinephrine	53.9 \pm 2.7	41.2 \pm 3.9 ^a	56.3 \pm 4.3	82.2 \pm 3.0 ^a
Synthesis of ^3H -norepinephrine	7.2 \pm 0.6	5.3 \pm 0.2 ^a	7.2 \pm 1.0	21.3 \pm 1.6 ^b
Synthesis of ^3H -dopamine	4.3 \pm 0.3	4.3 \pm 0.5	4.3 \pm 1.1	17.8 \pm 1.8 ^b
Epinephrine	402 \pm 39	209 \pm 23 ^b	410 \pm 53	326 \pm 26
Norepinephrine	213 \pm 26	149 \pm 15 ^c	195 \pm 19	185 \pm 24
Dopamine	2.5 \pm 0.3	7.1 \pm 0.8 ^b	2.1 \pm 0.4	4.9 \pm 0.7 ^c

Difference with control:

^a $p < 0.01$.

^b $p < 0.001$.

^c $p < 0.05$.

antioxidants as antiarrhythmics calls for a clearer notion of whether they only act directly on the heart or may have central action as well.

The direct cardiac action has now been demonstrated in numerous experiments. To give an example (Figure 16), a water-soluble hydroxypyridine antioxidant (2-ethyl-6-methyl-3-hydroxy-pyridine) added to the perfusing solution eliminates the spontaneous stable bigeminy of the isolated auricle. A large number of similar works available leave no doubt of the local cardioprotective ability of antioxidants. Their central action, however, has for a long time been much more questionable.

To elucidate this, we have studied the effect of ionol on the content and the synthesis of catecholamines and corticosterone in the stress reaction. The results proved somewhat unexpected. Table 10 presents the data on catecholamine metabolism in the adrenal glands. Besides the known fact that stress causes a discharge of epinephrine and norepinephrine whereby their content decreases in the adrenal medulla, ionol in stress rather surprisingly enhances severalfold the synthesis of dopamine and norepinephrine, preventing thus the decline in norepinephrine content in the adrenal glands; interestingly, despite greater synthesis with ionol, the dopamine content increases in a considerably smaller degree than without it. It appears that under the influence of ionol the adrenal glands become a source of dopamine.

TABLE 11
Effect of Ionol on Neuronal Uptake of ^3H -Norepinephrine and Synthesis of ^3H -Catecholamines ($10^3 \text{ cpm/g tissue}$) and on the Catecholamine Content ($\mu\text{g/g}$) in Rat Heart in Stress

Indices	Series (n = 12 each)			
	Control	Stress	Ionol	Ionol + stress
Neuronal uptake of ^3H -norepinephrine	70.7 \pm 4.5	48.6 \pm 3.5 ^a	74.6 \pm 4.1	130.0 \pm 11.3 ^a
Synthesis of ^3H -norepinephrine	7.4 \pm 0.5	5.2 \pm 0.6 ^b	9.3 \pm 1.4	34.3 \pm 0.4 ^a
Synthesis of dopamine	1.9 \pm 0.08	2.2 \pm 0.26	6.3 \pm 0.94 ^b	23.1 \pm 0.32 ^a
Norepinephrine	1.0 \pm 0.2	0.6 \pm 0.1 ^b	0.9 \pm 0.2	0.7 \pm 0.1
Dopamine	0.1 \pm 0.01	0.2 \pm 0.02 ^c	0.3 \pm 0.03 ^a	0.2 \pm 0.03 ^a

Difference with control:

^a $p < 0.001$.

^b $p < 0.05$.

^c $p < 0.01$.

Table 11 shows the data on catecholamine synthesis and content in the heart where they are known to be mainly localized in the adrenergic terminals. Ionol in stress can be seen to enhance dopamine synthesis tenfold and norepinephrine sixfold, and to lessen thus the decline in myocardial norepinephrine.

To appreciate the great activation of dopamine synthesis by ionol, it should be borne in mind that it is a central regulator of motor activity,⁶⁴ a vasodilator and natriuretic,⁶⁵ and at the same time has much influence on the stress reaction itself. Thus dopamine has been shown to suppress the ACTH-dependent adrenal synthesis of corticosterone⁶⁶ and therefore acts as a factor checking the hypophysial-adrenal link of the stress reaction.⁶⁶ In full conformity with this, we have found that ionol abolishes the elevation of corticosteroids in the adrenal glands and blood, which is commonly observed in stress or after administration of ACTH.⁶⁷

Thus, the antioxidant used by us is active both in the heart proper and in the regulatory mechanisms. In the final analysis, it augments the content of such a stress-limiting factor as dopamine, prevents catecholamine depletion, and (probably through dopamine) shuts down the hypophysial-adrenal link of the stress reaction.

There are no grounds for regarding ionol as some unique drug; on the contrary, it appears to be a general issue that dealing with any antioxidant we must consider its probable central action. At any rate, in our further work aimed at using the antioxidant to avert arrhythmias in man we took account of this central action when selecting the patients. The therapy was carried out in two groups. The first one comprised patients with neurocirculatory dystonia and neurasthenic syndrome, i.e., phenomena of cardiophobia, hypochondria, and depression; the physical load test ruled out ischemic disease, and arrhythmias occurred mostly at rest. The second group was patients with moderate angina of effort, with arrhythmias elicited under load and being clearly of ischemic origin.

Ionol was administered *per os* at 20 mg/kg daily for 10 to 12 d. Arrhythmias were assessed by 24-h monitoring and counting separately the number of ventricular and atrial extrasystoles for every 3 h. No positive effect was observed with angina patients, but it was quite certain in neurocirculatory dystonia (in essence — in neurogenic arrhythmias). Table 12 demonstrates the distribution of ventricular extrasystoles through the day before and after ionol treatment. As can be seen, the extrasystolia, which was most pronounced in these patients during the night, was markedly alleviated by the antioxidant; this quite reliable effect was maintained for at least 2 months after the end of the course. Simultaneously the manifestations of the neurasthenic syndrome and especially hypochondriac complaints and depressive phenomena were much abated.

TABLE 12
Effect of a Course of Ionol on the Mean Number of
Supraventricular (SVE) and Ventricular (VE) Extrasystoles
Throughout the Day in 21 Patients with Neurocirculatory
Dystonia

Time of day (h)	Mean number of extrasystoles			
	Before treatment		After treatment	
	SVE	VE	SVE	VE
00—03	651 ± 72	2156 ± 187	262 ± 37	1005 ± 95
03—06	342 ± 45	1058 ± 115	116 ± 29	651 ± 75
06—09	95 ± 10	938 ± 76	64 ± 9	405 ± 72
09—12	116 ± 11	451 ± 66	55 ± 7	156 ± 47
12—15	61 ± 7	578 ± 31	45 ± 6	256 ± 51
15—18	75 ± 8	296 ± 42	26 ± 5	105 ± 21
18—21	115 ± 10	415 ± 56	86 ± 7	156 ± 10
21—24	239 ± 23	851 ± 92	106 ± 9	211 ± 25

Thus, there is reason to believe that the antiarrhythmic action of ionol and other antioxidants in animals and man can take place both in the heart itself and through neural regulation.

Another possible and noteworthy application of antioxidants stems from the observation that the power of the natural antioxidant systems increases as the organism adapts to repeated stress and physical load (see above). On the strength of these data and in accordance with the traditions of our laboratory, we exploited the principle of mimicking the natural organismic stress-limiting systems, i.e., used the antioxidant ionol to improve physical endurance first in animals¹² and then in humans.⁵⁰

Assays of the peroxidation product pentane, in the air exhaled by subjects have shown that submaximal and maximal veloergometric (VEL) work is accompanied with an outburst of free-radical oxidation, which can be suppressed with antioxidants.

Figure 17 shows that a prior 10-d course of 20 mg ionol daily per kilogram body weight markedly diminishes the pentane content in exhaled air upon maximal VEL. Further, it turned out that checking the outburst of peroxidation under physical load is physiologically significant since it can appreciably enhance physical endurance. According to Table 13, at submaximal load to exhaustion the antioxidant increases the total performed work by more than 40% in untrained subjects and by 80% in trained athletes.

In the aggregate the data available mean that under physical load, activated free-radical oxidation imposes a limitation on the performance. The organism can alleviate this by enhancing the synthesis of antioxidant enzymes; on the other hand, the same can be achieved by introducing antioxidants into the organism. At present, the state of free-radical oxidation and of the antioxidant systems is known to change in adaptation to many environmental factors, and we have grounds for concluding that, on the one hand, environmental adaptation determines the antioxidant status and the free-radical homeostasis in the organism as a whole and in the heart in particular; on the other, exogenous antioxidants can be used for controlling and correcting the adaptive processes.

To conclude this section, mention should be made of some promising trends in developing the antioxidant protection of the heart and the organism in whole.

The first one is studying the role of pro-oxidants and antioxidants at the genetic level, and designing such antioxidants as would act not as simple free-radical traps, but as genomic inductors. Figure 18 shows that the major natural antioxidant α -tocopherol not only scavenges free radicals, but also induces the synthesis of enzymes responsible for formation of another group of natural antioxidants and electron carriers — ubiquinones.⁶⁸ This is not a unique

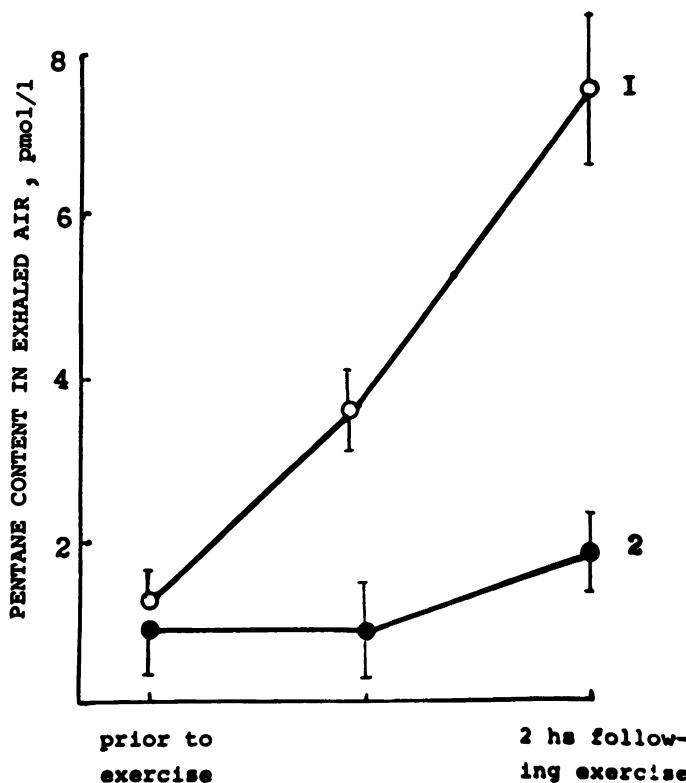


FIGURE 17. Effect of physical load and ionol on the pentane content in exhaled air in human subjects. (1) Before and (2) after administration of ionol.

TABLE 13
Effect of Ionol on Physical Endurance of Untrained and Trained Subjects

	Untrained (n = 6)		Trained (n = 24)	
	Before ionol	After ionol	Before ionol	After ionol
Duration of load, min	13.7 ± 1.9	19.6 ± 5.5*	23.8 ± 3.7	44.0 ± 9.6*
Work, N·m	2650 ± 422	3812 ± 637*	4666 ± 935	9245 ± 1920*

* Difference with initial indices significant at $p < 0.05$.

example: Wefers et al.⁶⁹ have reported that a synthetic antioxidant, butyl hydroxyanisole, is also a potent inductor of the antioxidant enzyme NAD(P)H: quinone reductase.

Another promising trend is elucidation of the interrelation between the antioxidant and other cell regulatory systems. Figure 19 demonstrates how the antioxidant status of the cell, in particular the myocyte of the coronary artery, can determine the balance of prostacyclin and thromboxane synthesis: in deficiency of antioxidant protection thromboxane formation prevails, with increasing probability of coronary spasm and thrombosis, whereas with optimal antioxidant protection the situation is opposite. There is good reason for studying further the relations of the cellular free-radical homeostasis with the prostaglandin (PG) system, the adenosinergic system, the receptor-dependent system of cyclic nucleotides.

The next approach of promise, to my mind, is combining antioxidants with other cardiac

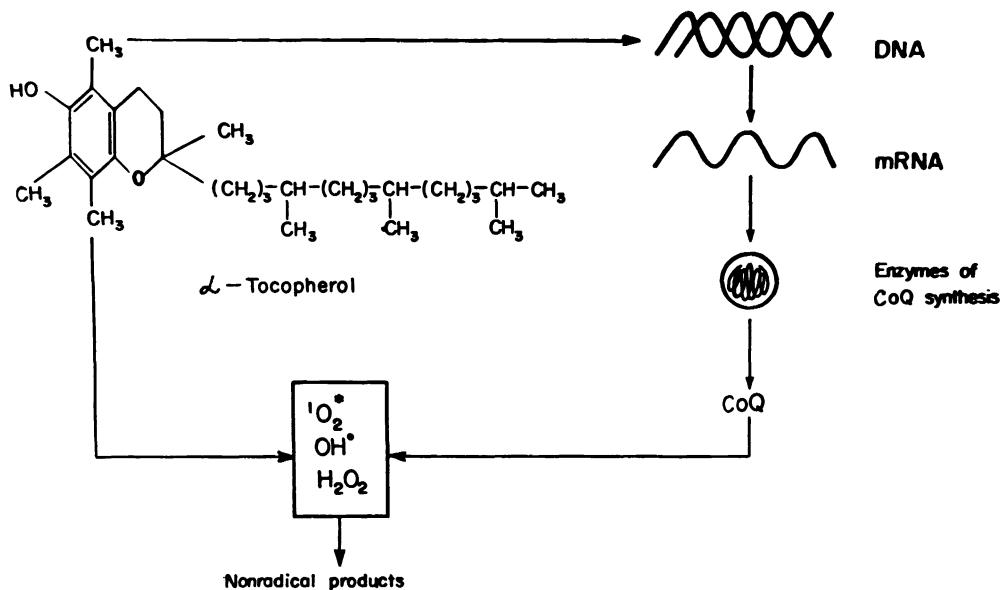


FIGURE 18. α -Tocopherol induces enzymes responsible for synthesis of ubiquinones. For explanations see text.

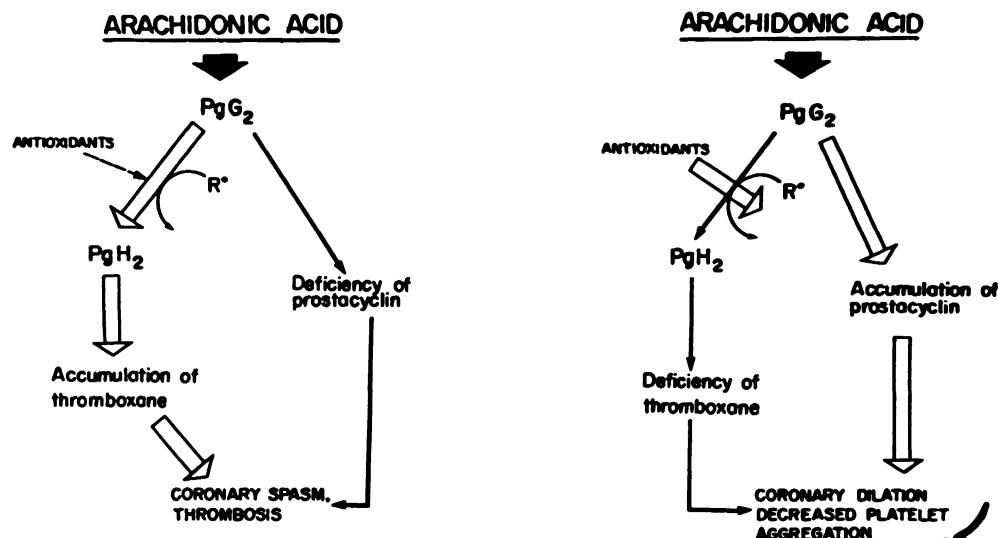


FIGURE 19. Effects of antioxidants on prostacyclin and thromboxane synthesis. PGG₂ and PGH₂, arachidonic acid endoperoxides, precursors of prostacyclin and thromboxane; R•, free radical. For explanations see text.

protectors; one of the numerous examples from this field concerns the vasodilatory and antiarrhythmic action of adenosine. Its mechanism is shown in Figure 20, yet at the same time it can be seen that the breakdown of adenosine may create a surplus of substrate for xanthine oxidase and thereby enhance generation of free radicals. It is therefore understandable that in some cases adenosine, especially in combination with inosine, may promote reperfusion arrhythmias or at any rate be not helpful against these arrhythmias. Hence it is expedient to combine the adenosine derivatives with antioxidants, e.g., of the ubiquinone group.

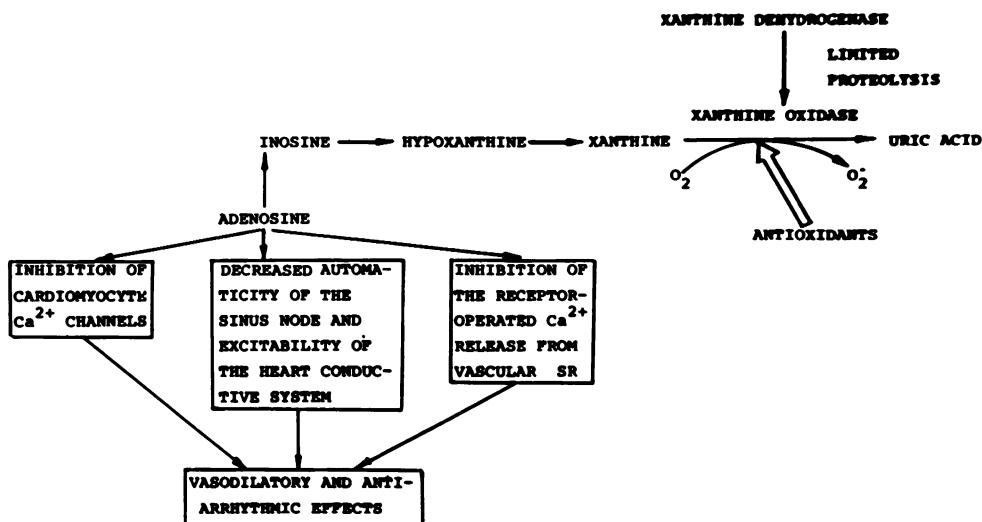


FIGURE 20. Vasodilatory and antiarrhythmic action of adenosine and the role of antioxidants in potentiating this action. For explanations see text.

TABLE 14
Effects of Coenzyme Q₁₀ and Cyclohexyl Adenosine on the Mean Duration of Grave Ventricular Arrhythmias (Ventricular Tachycardia plus Ventricular Fibrillation)

	Duration of arrhythmias (s)	
	Ischemia (10 min)	Reperfusion (10 min)
Control (n = 12)	150 ± 37	371 ± 57
Cyclohexyl adenosine 0.1 mmol/kg (n = 12)	33 ± 16 ^a	359 ± 104
Coenzyme Q ₁₀ 10 mg/kg (n = 10)	121 ± 30	124 ± 26 ^b
Q ₁₀ plus cyclohexyl adenosine (n = 12)	26 ± 12 ^b	123 ± 93 ^c

Difference with control:

^a $p < 0.02$.

^b $p < 0.01$.

^c $p < 0.05$.

Table 14 presents the results of treating ventricular arrhythmias in experimental acute cardiac ischemia and reperfusion. In ischemia, cyclohexyl adenosine decreases their duration fivefold, but has no effect whatever on reperfusion arrhythmias, and does not decrease animal mortality from such arrhythmias. Conversely, coenzyme Q₁₀, does not appreciably affect the ischemic arrhythmias, but decreases the duration of grave reperfusion arrhythmias almost fourfold and mortality threefold. When the agents are used together they are not synergistic, but an indisputable advantage of such a combination is that it is equally effective against ischemic and reperfusion arrhythmias. This may be not the most efficient combination possible, but is a most definite evidence of the prospects in this field.

Finally, the available, means of assaying the peroxidation processes in the intact organism (pentane assays in the exhaled air in various functional tests, assays of antioxidant enzymes, and other more direct approaches) appear to open the possibility of a standardized clinical evaluation of antioxidant status of the organism, which can be of certain practical importance in various diseases. Thus, our preliminary studies have shown that patients with pronounced

IHD risk factors often have lowered blood levels of antioxidant enzymes and respond to physical load with enhanced pentane production. It cannot be excluded that after a thorough analysis a reduced antioxidant status will be entered into the list of the risk factors of ischemic disease.

On the whole, the antioxidant systems of the heart itself and the use of exogenous antioxidants show much promise for heart protection.

III. PROSTAGLANDIN SYSTEM AND HEART PROTECTION FROM STRESS AND ISCHEMIC DAMAGES*

Under extensive study at present is the group of arachidonic acid derivatives, or eicosanoids, which participate as modulators in regulating the functions of various organs and tissues, from vascular tone and blood clotting mechanisms to inflammation processes and release of neuromediators and hormones.

A special place in this group is occupied by PG. Unlike other eicosanoids, PGs are capable of restricting and modulating the activation of the adrenergic link of the stress reaction, and also exhibit a cytoprotective action in the heart and other organs, which allows the PG system to be classed with the stress-limiting systems of the organism.

The initial step in formation of PG (and other eicosanoids) is the liberation of arachidonic acid from cell membrane lipids and to some extent from other lipids.⁷⁰ Formation of free arachidonic acid triggers a "cascade" of eicosanoid synthesis involving a number of enzymes such as prostacyclin synthetase, thromboxane synthetase, endoperoxide isomerase, and endoperoxide reductase.^{71,72} It should be noted that some or other eicosanoids are preferentially produced depending on the type of cell. Thus, in blood platelets mostly thromboxanes are formed, while the yield of PG is very low,⁷³ whereas myocardium most typically produces prostacyclin (PGI_2)⁷⁴ and PG of the E group (PGE_1 , PGE_2).⁷⁵ The coronary vascular wall yields mainly PGI_2 .^{73,76}

It should also be emphasized that preferential formation of particular eicosanoids in the "arachidonic cascade" depends on a number of factors affecting the steps of this cascade. Thus, blocking the lipoxygenase pathway gives rise mainly to PGs which are cytoprotective and vasodilatory, while leukotrienes which act in an opposite way are not formed, etc. It is noteworthy that certain steps of the cascade are associated (as already mentioned in the first section of this chapter) with LPO activation and lipid peroxide formation and are susceptible to antioxidants, and some enzymes in the cascade are sensitive to these peroxides.⁷¹ It can thus be thought that during the stress reaction PG synthesis and the scale of their protective effect are in great measure determined by the activity of the antioxidant system and by the LPO level.

The PG system is in a certain way coupled with the stress-effecting systems: when neuromediators and stress hormones stimulate the cell functions (see Chapter 1), they may simultaneously activate eicosanoid synthesis by promoting the liberation of arachidonic acid.

Indeed, hormone and mediator action on the cell Ca^{2+} -mobilizing receptors results in activation of phospholipase C that hydrolyzes phosphatidylinositol (PI) and its derivatives to produce diacyl glycerol (DAG) and inositol triphosphate (IP_3). Thereupon DAG, on the one hand, acts together with IP_3 to raise the cytosolic Ca^{2+} and activate the cell functions, and on the other is catabolized with appropriate lipolytic enzymes to liberate arachidonic acid. Again, elevated Ca^{2+} activates the phospholipase C isozyme cleaving other membrane phospholipids (and thereby producing DAG and further arachidonic acid) and phospholipase A₂, which is the major enzyme liberating arachidonic acid from any phospholipids and phosphatidic acid.^{70,77,78}

It should also be noted that enhancement of cell functions by agonists of the adenylate

* With M. G. Pshennikova.

cyclase receptors is also coupled with liberation of arachidonic acid through phospholipase A₂ activation by the cAMP-dependent protein kinase. In the heart, activation of PGE₂ and PGI₂ synthesis by catecholamines can be suppressed not only with blockers of the Ca²⁺-mobilizing α-receptors, but also with a β-adrenoreceptor blocker.⁷⁵

Thus, it can be supposed that during the stress reaction the increased output of hormones and mediators that are agonists of Ca²⁺-mobilizing and adenylate cyclase-activating receptors not only changes the cell function, but at the same time can, through liberation of arachidonic acid, stimulate the biosynthesis of eicosanoids and among them PG.

The actual existence of such coupling has been to date well proved for catecholamines. There are three main lines of evidence: (1) catecholamines increase the PG output into the blood from various tissues; (2) the catecholamine-induced increase in PG output is blocked by inhibitors of catecholamine action; (3) the catecholamine-induced increase in PG output is blocked by inhibitors of PG synthesis. These data have been obtained for different organs; we shall only consider some of them pertaining to the heart.

In the late 1970s to early 1980s it has been shown that sympathetic stimulation of the isolated rabbit heart with intact innervation entails marked release of PGE and PGI₂ into the perfusate, which can be prevented by blocking the cardiac adrenoreceptors with propranolol and phentolamine.^{74,79} With canine heart *in situ* administration of epinephrine and norepinephrine greatly increased the PGE content in venous blood flowing off the heart; this was completely abolished by indomethacin, which blocks cyclooxygenase, a key enzyme of the "arachidonic cascade".⁸⁰ Inhibition of the catecholamine-induced output of PG by indomethacin was also observed with the rabbit heart.⁷⁹ Finally, ¹⁴C-labeled arachidonic acid has been used to demonstrate that the adrenergically stimulated PG output from the heart is the direct consequence of their enhanced synthesis.⁸¹

It has also been found that vasopressin activates PGI₂ synthesis in the vascular wall⁸² and angiotensin II activates it in the adrenal glands.⁸³

In the context of this exposition it is essential that the catecholamine-activated PG system can, through feedback, modulate the adrenergic effects on the heart and other organs by affecting norepinephrine release from sympathetic terminals and catecholamine action on the target organ cells. Most of the data available indicate that PGE₁, PGE₂, and PGI₂, usually produced by activated sympathetic regulation in the number of organs, are physiological modulators of sympathetic transmission, limiting excessive release of norepinephrine from sympathetic terminals; PGs do not affect norepinephrine reuptake, so the amount of the mediator in the action zone decreases and its influence on the effector cells is restricted correspondingly (for references see Wennmalm et al.)⁷⁵

It is also important that PGs produce their regulatory action directly at the level of the effector cells as well. In particular, PGI₂ synthesized in the coronary vascular endothelium and intima is a coronarodilator (especially in small arteries) and a potent inhibitor of platelet aggregation, unlike thromboxane A₂ (TXA₂) produced in the platelets, which is a powerful inductor of platelet aggregation and vasoconstriction,^{73,76} and unlike leukotrienes synthesized mainly by the neutrophils and having a pronounced coronaryconstrictive action.⁸⁴

PGI₂ acquires special significance in coronary failure. In patients with coronary disease, inhibition of PGI₂ synthesis by indomethacin has been shown to attenuate the coronary sinus flow and increase the resistance in large coronary arteries despite enhanced oxygen consumption, whereas in normal coronary arteries indomethacin does not substantially reduce their diameter.⁷⁶ This phenomenon appears to be due to the prevalence of leukotrienes and their vasoconstrictive action, since there are data that show leukotriene synthesis is enhanced in coronary failure and ischemia.⁸⁷ It has also been shown that spontaneous reduction of coronary flow in partial constriction of the coronary artery, which is caused by platelet aggregation, develops as a result of the lack of PGI₂ and decreasing PGI₂/TXA₂ ratio, and is completely prevented by administration of PGI₂.⁷³

Recently, a similar protective action has been found with PGE₁, which upon intracoronary infusion prevented reocclusion of coronary arteries in dogs with thrombosis of the circumflex coronary artery produced with dosed local application of electric current causing a 50% reduction of the arterial lumen; the PGE₁ effect was due to inhibition of platelet aggregation and its vasodilatory action.⁸⁵

PGs operate through specific binding sites on the target cell membranes.^{86,87} Besides, there is evidence for their cytoprotective action associated with direct influence on the cell membranes that increases membrane resistance to damaging factors. In particular, increased PGE content in gastric mucosal cells has been shown to prevent injury from concentrated solutions of ethanol, mannitol, or hydrochloric acid.⁸⁸

In a study of the influence of PGI₂ and its stable synthetic analog quinolin-169 on isoproterenol cardiac damage, administration of PG before isoproterenol substantially attenuated the size of myocardial necrosis. Since the maximal protective effect was observed when PG was administered 1.5 to 2 h before (and within this time PGs are completely broken down in the organism), the authors concluded that the protective effect was underlain by the stabilizing action of PGs on the cardiomyocyte membranes.⁸⁹

The mechanism of this cytoprotective action of PGs appears to be related to their lipid nature and their influence on the membrane lipid bilayer. Indeed, it has been recently⁹⁰ shown that administration of a PGI₂ analog iloprost 20 min after coronary artery ligation, when any other PG effects are unlikely to be of any help, appreciably attenuates in rats the ischemic destruction of cardiomyocyte membranes and loss of phospholipids and creatine kinase.

Hence, PGs can be supposed to prevent stress damages or diseases provoked by the stress reaction. The protective effect of PGs is most definite in stress ulceration of the gastric mucosa. Such lesions produced in rats by a 2-h immobilization stress are efficiently averted with prior administration of PGE.⁹¹ Recently, the development of gastric stress ulcers has been found to depend on the endogenous PG level in the gastric mucosa. Ulceration in immobilization⁹² and other⁹³ types of stress is associated with a decline in PGE₂, PGI₂, *et seq.* in the mucosa. Prior activation of PG synthesis by intragastric administration of arachidonic acid prevents both the PGE₂ drop and ulceration.⁹² It has also been shown that the known antiulcerous action of the histamine H₂-receptor blocker famotidine is due to the fact that the drug prevents the stress-induced decline in gastric mucosal PG.⁹³ PGs provide protection against such damages by preventing gastric vasoconstriction owing to their vasodilatory properties, and by stabilizing the gastric cell membranes.

Of greatest interest for us is the role and possible protective action of PGs against stress-induced and ischemic damage to the heart. Most of the works of recent years testify that PGs display preventive and protective effects in disturbances to cardiac structure and contractility caused by stress and ischemia. Thus, we⁹⁴ have shown that reduction of myocardial extensibility and contractility after immobilization stress is prevented by prior administration of PGE₂ to animals; administration of indomethacin to inhibit PG synthesis, conversely, aggravated the stress damage to myocardial function. With the isolated rabbit heart, addition of PGI₂ to the perfusing solution starting from 10 min prior to left coronary artery ligation prevented the ischemia-induced "discharge" or norepinephrine from sympathetic terminals, as well as the drop in ATP and the creatine phosphokinase (CPK) leakage from the myocardium.⁹⁵ It can be supposed that in both works the protective effect of PG is mostly due to checking the excessive release of norepinephrine and therefore the possible adrenergic injury to the myocardium. At the same time, the above-discussed properties of PGs suggest that their coronarodilatory and cytoprotective features are also involved.

More direct evidence for this has been obtained in studies where PGs were administered not before, but after coronary occlusion, in already existing ischemia. Thus, in cats introduction of a PGI₂ analog iloprost in reperfusion 30 min and 2 h after the onset of ischemia

prevented the drop in CPK activity and leakage of cathepsin D from the ischemic zone and substantially enhanced the coronary flow in the nonischemic myocardium, without changing the infarct size;⁹⁶ the authors regard these PG effects as the cytoprotective ones. The cytoprotective, membrane-stabilizing action of PG has been convincingly proved in the work⁹⁰ where a stable PGI₂ analog was administered to rats 20 min after coronary occlusion; the preparation markedly attenuated the loss of phospholipids caused by ischemic damage to cardiomyocyte membranes.

The ability of PGs to restrict the sympathetic influences and their cytoprotective properties are apparently the basis of their antiarrhythmic action in ischemic arrhythmias where, as already more than once mentioned, the stress reaction is pathogenetically important. Thus, it has been shown⁹⁷ that ligation of the left anterior descending coronary artery in anesthetized dogs led to 35% mortality within 6 h; no mortality was observed among animals receiving PGI₂ (0.32 µg/kg·min throughout the experiment). In the process, PGI₂ affected neither the infarct size nor the hemodynamic indices measured 15 and 45 min after the onset of ischemia. The authors⁹⁷ were of the opinion that the protective effect of PGI₂ was due to prevention of lethal arrhythmias. In a study with ECG recording in conscious dogs,⁹⁸ occlusion of the left circumflex coronary artery caused ventricular fibrillation in 53% (9/17) and death with 80 min in 64% of the control group; in animals receiving PGI₂ (0.1 µm/kg·min) the incidence of ventricular fibrillation was only 6% (1/17) and mortality was threefold less.

In line with these data are the results of works^{99,100} showing that development of arrhythmias correlates with a decline in the PGI₂ content and the PGI₂/TXA₂ ratio. When the platelet-activating factor (PAF), which is released from leukocytes in ischemia and stress and promotes production of leukotrienes and TXA₂, was administered to guinea pigs, it substantially lowered the threshold of ouabain-induced arrhythmias and increased their lethality. This arrhythmogenic effect of PAF could be completely abolished with a selective thromboxane receptor antagonist BM 13.177 and less efficiently with the lipoxygenase inhibitor esculetin.¹⁰⁰ At the same time aspirin, which inhibits a key enzyme of PG and thromboxane synthesis, cyclooxygenase, did not have much influence on the PAF effect, but still showed a tendency to raising the threshold of arrhythmias, probably by attenuating the TXA₂ synthesis. Thus, in arrhythmogenic situations the PG/thromboxane ratio comes to be of great importance.

There is yet no full understanding of the localization of the antiarrhythmic effect of PG. In the glycoside model of arrhythmias, the antiarrhythmic effect is unrelated to their central action: administration of PGI₂, PGE₂, and PGF_{2α} into brain ventricles was ineffective against ouabain-induced extrasystoles and fibrillation, though intravenous infusion suppressed these cardiac rhythm disorders, whereas the β-blocker propranolol was effective in both cases.¹⁰¹ The genesis of glycoside arrhythmias involves enhanced sympathetic influence on the heart,¹⁰² hence it can be supposed that PGs restrict it by hindering the norepinephrine release from sympathetic terminals in the myocardium. Again, since glycoside arrhythmias involved interruption of the membrane cation pumps, the membrane-stabilizing action of PGs may also contribute to their protective effect. Further, PGs can stimulate the peripheral parasympathetic regulation of the heart which produces an antiarrhythmic effect in glycoside intoxications.¹⁰¹

In the aconitic model, on the contrary, the antiarrhythmic effect of PGs appears to be central (since it takes place upon central administration).¹⁰³

It must be accentuated that the very question of the use of exogenous PGs in cardiac ischemic damage and arrhythmias is quite complicated and requires further serious study, because besides the above and other numerous data on the protective role of PGs there are reports^{104,105} on their possible detrimental action: PGE₂ and PGI₂ can aggravate, in a dose-dependent way, the functional disturbances and arrhythmias in the isolated heart exposed to ischemia or glycosides, which is believed to result from activation of the slow Ca²⁺ channels, overloading of the cardiac cells with calcium, and mitochondrial damage.¹⁰⁴

At the same time, there is a considerable body of evidence that a natural increase in the power of the PG system in the organism and normalization of the relations between PGs and other eicosanoids enhance the resistance of the organism to injurious influences. Thus, it has been convincingly demonstrated that reinforcement of the PG system in the gastric mucosa either with precursors of PG synthesis⁹² or with prior adaptation to stress⁸⁸ prevents the PG deficiency and ulceration in stress⁹² and increases the resistance of the gastric mucosa to chemical injury.⁸⁸

Atherogenic dyslipoproteinemia is known as a basic cause of atherogenesis and atherosclerosis, a heavy risk factor of IHD and myocardial infarction. In recent years the blood high-/ and low-density lipoproteins (HDLP/LDLP) ratio has been found to be rather clearly related with the vascular PG synthesis. (May it be recalled that atherogenic dyslipoproteinemia evoked by stress or other causes results from impairment of the hepatic cholesterol-catabolizing enzymes and lecithin:cholesterol acyltransferase involved in HDLP formation.^{106,107}) Over a decade ago, development of atherosclerosis has been shown to combine with PGI₂ deficiency.¹⁰⁸ Recently, certain patients with ulcerous disease of the digestive tract have been found to have hypercholesterolemia and atherogenic dyslipoproteinemia; treatment of ulcers with the PGE₂ analog enprostil was accompanied with normalization of the cholesterol status.¹⁰⁹ These results were supported by a special study of healthy subjects where the PGs decreased LDLP cholesterol and increased HDLP cholesterol.¹⁰⁹

We¹¹⁰ have shown that prior adaptation to stress elevates the blood PGE; simultaneously, such adaptation prevents the stress-induced atherogenic dyslipoproteinemia.¹⁰⁶ Hence, it can be concluded that PGs are capable of normalizing the lipoprotein metabolism. Since hepatocyte membrane damage is important in the pathogenesis of atherogenic dyslipoproteinemia, this protective action of PGs can be attributed to their cytoprotective effect in the liver. On the other hand, there are recent data¹¹¹ that HDLP stimulate while LDLP suppress PGI₂ synthesis in isolated coronary vessel microsomes. This implies a vicious circle: atherogenic dyslipoproteinemia may suppress the vascular PG synthesis, which in its turn reduces their hepatoprotective capacity and thereby aggravates the dyslipoproteinemia. It can thus be thought that increasing the power of the PG system enhances hepatocyte resistance to damaging factors including stress, and can therefore be a means of preventing atherosclerosis.

In the aggregate, the data available suggest that, first, increased power of the PG system plays a certain part in the protective effect of adaptation to stress against the stress-induced and ischemic disturbances to cardiac rhythm and function, and second, there is certain promise in studying the cardioprotective properties of PGs and their analogs with a view to their use against arrhythmias.

IV. ADENOSINERGIC SYSTEM AND HEART PROTECTION FROM STRESS, ISCHEMIC, AND REPERFUSION DAMAGES*

There are three main features by which the adenosinergic system (i.e., adenosine and its receptors) can be classed with stress-limiting systems. First, adenosine has pronounced vasodilatory activity,^{76,112} is not normally found in blood and peripheral tissues in acting concentrations, but is formed in the heart and other tissues and released into the blood in conditions associated with deficit of creatine phosphate (CP) and ATP and enhanced oxygen consumption in the cells: ischemia, hypoxia, physical load, action of catecholamines and hormones, etc.^{112,115,117} This means that the stress reaction is naturally coupled with activation of the adenosinergic system. Second, adenosine can restrict the release of adrenergic mediators both in the central nervous system and in the periphery.¹¹³⁻¹¹⁵ Third, it can modulate the action of neuromediators and hormones on the target organ cells.^{112,116-118} Here we shall focus on these features of adenosine as applied to the heart and vessels.

* With M. G. Pshennikova.

In the myocardium, adenosine is produced from 5'-AMP by 5'-nucleotidase bound with the cardiomyocyte membrane. The enzyme is inhibited by ADP, AMP, and most strongly by CP, so normally its activity in the cell is about two orders of magnitude lower than *in vitro*; it is activated by free Mg²⁺. Adenosine is catabolized by adenosine deaminase to inosine, this reaction being inhibited by its product.^{112,119}

In a stress reaction evoked by load, hypoxia, or otherwise, under the influence of excess catecholamines and hormones and enhanced cardiac function, myocardial consumption of ATP and CP outstrips their resynthesis so that the level of these inhibitors of 5'-nucleotidase declines while the amounts of its activator Mg²⁺ (released on breakdown of ATP) and its substrate AMP increase, thus favoring formation of adenosine. The same chain of events naturally takes place in adrenergic and hormonal coronary constriction and in coronary thrombosis attending the stress reaction, especially in IHD and atherosclerosis.

It is noteworthy that catecholamines and other β- and α-adrenoreceptor agonists are themselves highly conducive to adenosine production in the heart in normoxia and ischemia.^{117,120} Adenosine is released from the myocardium into the interstitial space, reaches the coronary arterioles, and acts there to restrict the detrimental action of the very factors that stimulated its formation. In other words, there is a feedback mechanism.

Two effects of adenosine are of chief importance. The first one is coronary dilation, which counteracts vasoconstriction, enhances the coronary blood flow, and thereby facilitates oxygen and substrate supplies to the myocardium and normalizes the energy balance therein. The second one is checking the excessive adrenergic influence on the cardiac muscle, which attenuates its contractile function and oxygen consumption (thus also normalizing the energy balance) and prevents the cardiotoxic action of catecholamines.

The vasodilatory effect of adenosine has been convincingly proved in experiments on animals and in clinical studies.¹²¹⁻¹²⁴ In particular, administration of adenosine prevents the “no-reflow” phenomenon occurring in reperfusion after ischemia, and thus prevents or substantially attenuates myocardial damage in reperfusion.¹²³

Adenosine exerts its vasodilatory effect mainly by direct action on vascular myocytes, partly by interacting with the endothelium, and by suppressing the release of norepinephrine from the sympathetic terminals innervating the vessels. Now we know that the key role in contraction of the vascular smooth-muscle walls is played by the increase in calcium concentration in the myocytes owing to its entry from the outside and release from the intracellular depots. It is these mechanisms that are involved in the vasoconstrictive action of catecholamines and hormones,^{77,125} and there are grounds for believing that they are also affected in the vasodilatory effect of adenosine.^{76,116,126} This concept is demonstrated by the scheme in Figure 21. As can be seen, contraction of a vessel such as a coronary artery results from a chain of events triggered by increasing cytosolic Ca²⁺. Calcium enters the cell from the outside through potential-dependent channels (II) activated upon depolarization and through receptor-dependent channels (I) activated by hormones, and is released from the SR under the influence of the inositol phosphate circuit which is activated when norepinephrine from the sympathetic terminals acts on α₁-adrenoreceptors. The last two pathways are believed to be suppressible by adenosine,^{76,116,126} which is denoted by the “minus” signs in Figure 21. Though little is known as yet about the molecular mechanisms of these effects, they are likely to involve interaction of the adenosine receptors¹²⁷⁻¹²⁹ with the Ca²⁺ channels and adrenoreceptor complexes through the G-proteins.¹²⁹ An illustration of the possible adenosine receptor-adrenoreceptor interaction is a recent study¹¹⁶ with rat aortic strips showing that adenosine, while not affecting the calcium homeostasis and IP₃ content in the vascular myocytes at rest, largely checks the IP₃ synthesis and Ca²⁺ release from the SR stimulated by norepinephrine via the α₁-adrenoreceptors, and thereby prevents the norepinephrine-induced constriction of the vascular muscle. This effect is blocked with 8-phenyl theophylline, which is a selective blocker of the adenosine A₁ receptor, and potentiated with dipyridamole, which inhibits adenosine reuptake and thus sustains its effective concentration.

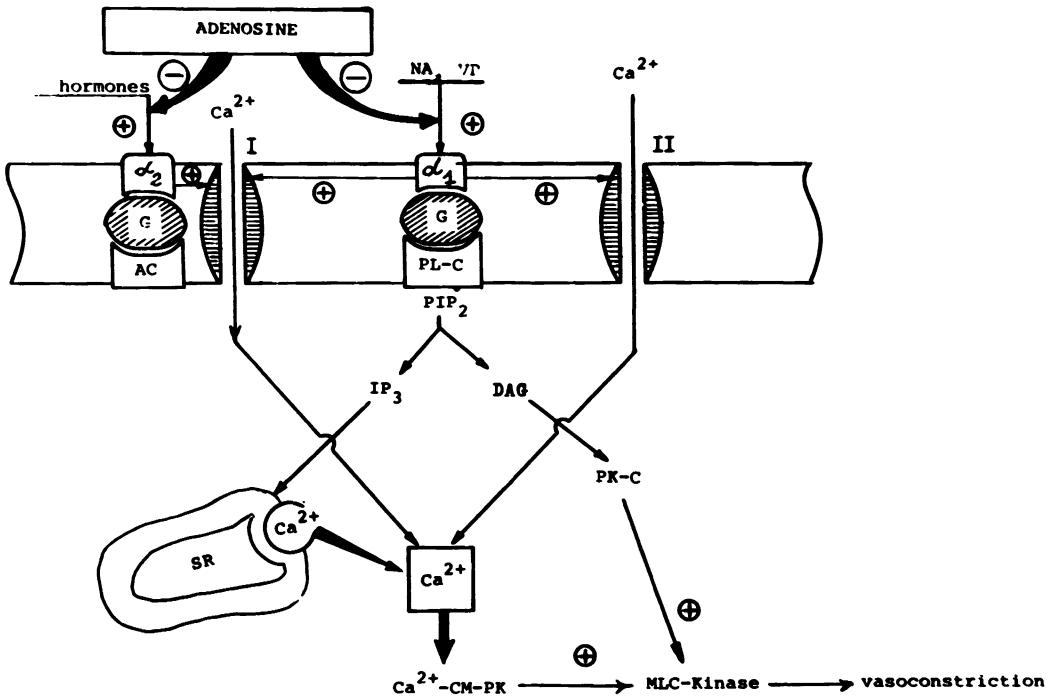


FIGURE 21. Vasodilatory action of adenosine on the stress-induced regulatory vascular spasm. (α_1 , α_2), Adrenoreceptors; (I) receptor-dependent Ca^{2+} channel; (II) potential-dependent Ca^{2+} channel; (AC) adenylate cyclase; (PL C) phospholipase C; (NE) norepinephrine, (VP) vasopressin; (PIP_2) phosphatidylinositol diphosphate; (DAG) diacyl glycerol; (SR) sarcoplasmic reticulum; (PK C) protein kinase C; ($\text{Ca}-\text{CM PK}$) calmodulin-dependent protein kinase; (MLC kinase) myosin light chain kinase; (G) G-protein of receptor complexes. For explanations see text.

As to the interaction of adenosine with the vascular endothelium and its vasodilatory effect,⁷⁶ its nature is poorly studied, but the data that adenosine definitely (and almost as efficiently as catecholamines) stimulates PGI_2 synthesis in the isolated heart,⁷⁵ i.e., actually in coronary endothelial cells, suggest that the endothelium-dependent vasodilatory action of adenosine may involve PGs.

The second aspect of adenosine, as stated above, is its ability to restrict the influence of adrenergic regulation on the heart. Firstly, adenosine suppresses norepinephrine release from sympathetic terminals in the heart through the presynaptic A_1 receptors.^{115,130} Secondly, it attenuates the positive inotropic^{117,126,132,133} and chronotropic¹³¹ effects of catecholamines on the heart.

As regards the latter, it was initially supposed that adenosine inhibits adenylate cyclase and cAMP synthesis.¹³² However, further studies revealed that attenuation of the catecholamine positive inotropic effect by adenosine may be accompanied by a lesser,^{117,132} the same, or even greater rise in cAMP (see Böhm et al.¹²⁶ and references therein). At the same time, adenosine and agonists of its receptors were found to suppress the positive inotropic effect of agents promoting cAMP formation or hindering cAMP breakdown, but not the positive inotropic effect of elevated extracellular calcium.¹²⁶ In other words, adenosine affects only those effects that are related to the cell cAMP content. Another line of research conducted with isolated ventricular myocytes¹³⁴ and papillary muscles^{135,136} showed that adenosine inhibits the catecholamine-induced Ca^{2+} entry into the cell and checks the rise in the action potential. Thus, a concept emerging¹²⁶ is that adenosine restricts the catecholamine action on the contractile myocardium not at the step of adenylate cyclase activation and cAMP synthesis, but somewhere later in the regulatory cAMP circuit. Adenosine is supposed to

somehow hinder the phosphorylation of the Ca^{2+} channels by cAMP-PK and to prevent thereby the rise in cell calcium, the development of the inotropic effect, and the possible cardiotoxic effect of excess calcium in exposure to catecholamines. A recent study¹³⁶ of the influence of several drugs on ischemic contracture in isolated rat heart supports the idea that adenosine produces its protective effect through a calcium-related antiadrenergic mechanism.

Adenosine can control the action of catecholamines not only in the contractile myocardium, but also at the level of pacemakers, suppressing the adrenergic tachycardia. Thus, in the isolated guinea pig heart with retained sympathetic innervation from the right stellate ganglion, exogenous adenosine restricts the sympathetically induced increase in the heart rate;¹³¹ this is still more pronounced in moderate hypoxia causing additional liberation of endogenous adenosine, and does not take place in the presence of 8-phenyl theophylline, which blocks the adenosine receptors.

Besides restricting the adrenergic influence on the pacemaker (as shown above for the contractile myocardium), adenosine can somehow act directly on the cells of the pacemaker and the conduction system, decreasing the sinoatrial node automatism, slowing down the spread of excitation through the atrioventricular node, and even causing atrioventricular block, as shown in experiments with guinea pig and rat isolated hearts and sinoatrial nodes.¹³⁷⁻¹³⁹ The mechanism is still obscure, but the similarity of this effect to that of acetylcholine has raised a suggestion¹³⁷ that adenosine may cause K^+ efflux from and hyperpolarization of the pacemaker cells. This opens some prospects of using activation of the adenosinergic system to rectify the cardiac rhythm disorders.

By and large, the adenosinergic system is a powerful cardiac and vascular self-regulatory mechanism capable of controlling the stress-induced and ischemic damage to the cardiovascular system, mainly by checking the excessive activation of the adrenergic regulation.

This concept is illustrated by the tentative scheme in Figure 22. The stress reaction, elicited by various factors including myocardial ischemia, and ensuing adrenergic activation result in depletion of the energy-rich phosphate compounds in the myocardium, which promotes liberation of adenosine. Adenosine suppresses the release of norepinephrine in the heart as well as checks the elevation of calcium in the coronary vascular myocytes (producing coronary dilation) and in the myocardiocytes (restricting the positive inotropic and chronotropic effects). This prevents or attenuates the detrimental effects of the lack of energy and calcium overloading in stress and/or ischemia. Of course the actual picture may prove much more complex than shown in the scheme. Here we only wanted to highlight the basic coupling between the stress-effecting adrenergic and the stress-limiting adenosinergic systems in the regulation of the heart.

At the same time it is important to remember that adenosine not only modulates the regulation of the executive organs, but is also active in the central nervous system as well, where it is present at high concentrations and its receptors and binding sites are abundant in various brain regions: hippocampus, cerebellum, hypothalamus, etc.^{114,115,134} At present the adenosinergic system is regarded as a chief modulator of neuronal function. It mainly acts to impede neurotransmission by suppressing the release of neuromediators through the A_1 and A_2 receptors located on the axonal presynaptic membrane.^{113,114,127,140} Besides, neuronal inhibition by adenosine has also been observed at the postsynaptic membrane level.^{127,140} These data come from experiments with exogenous adenosine and its receptor agonists; however, convincing evidence is available that the same holds true for the endogenous mechanism (see Jackisch¹¹³ for references).

Many researchers are of the opinion that both A_1 and A_2 receptors are coupled to adenylate cyclase; engagement of A_1 suppresses the enzyme and therefore Ca^{2+} entry through the neuronal presynaptic membrane, thus blocking the release of mediator; A_2 , conversely, activates the cyclase, Ca^{2+} entry, and mediator release.^{114,141} However, A_2 requires agonist concentrations three orders of magnitude higher than A_1 , i.e., micromolar rather than na-

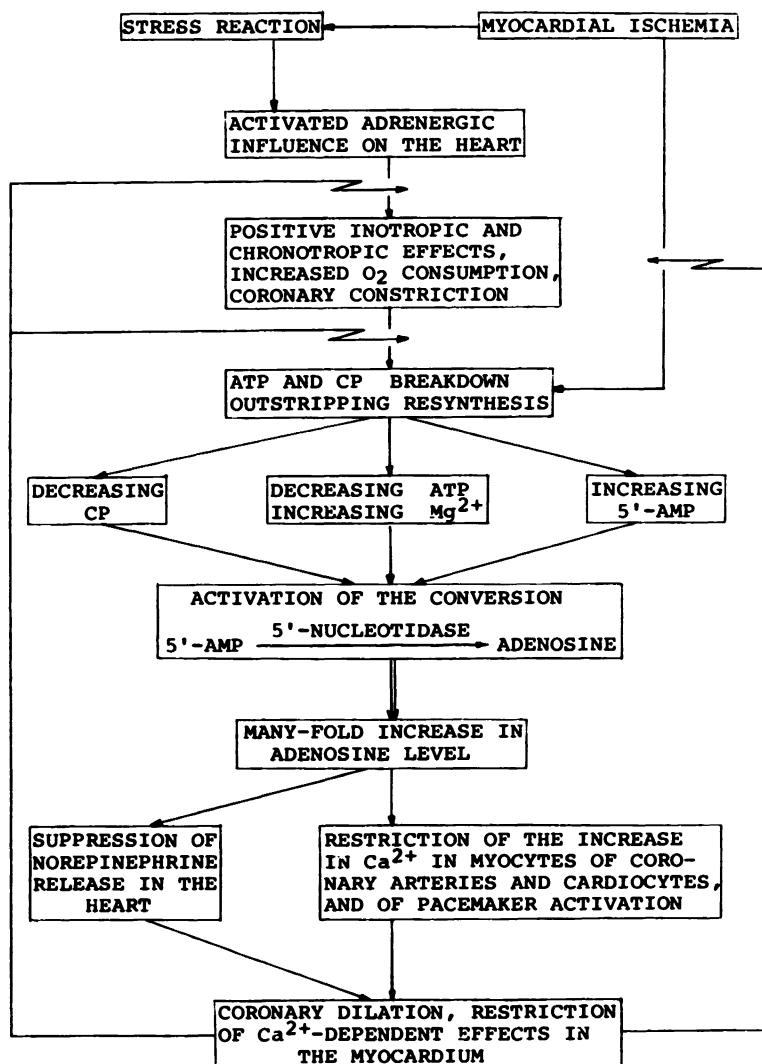


FIGURE 22. Coupling of the adenosinergic system to the stress reaction, and the cardioprotective effect of adenosine. For explanations see text.

nomolar levels (see Anderson et al.¹²⁷ for references). This apparently explains the mostly inhibitory, depressant action typical of adenosine in the central nervous system.¹¹⁴ On the other hand, there are data that show adenosine can act through its receptors directly on the Ca²⁺ channels of the neuronal membrane regardless of the adenylate cyclase activity.^{114,140} Taking account of what has been said above of the adenosine mechanisms in the heart and vessels, one can think that in the neurons adenosine affects the calcium homeostasis in much the same way, through receptor interaction with the Ca²⁺ channels and intracellular stores.

For us here it is essential that the central action of adenosine may be a weighty component of its stress-limiting function. Besides the data on inhibition of norepinephrine release in the brain, this idea is also favored by the fact that agonists of adenosine receptors and blockers of adenosine reuptake in the brain produce strong inhibition of the cardiovascular sympathetic tone and, in particular, have an antihypertensive effect.¹¹⁴ Further, adaptation to repeated brief exposures to immobilization and electric pain stress, which is an important

factor restricting the stress reaction and its injurious effects,² is accompanied by an increase in the number of A₁ receptors in the hypothalamus.¹²⁷

Thus, there are grounds for believing that increased power of the adenosinergic system may greatly contribute to enhancing the resistance of the organism to stress and ischemic damage. This can be attained with adaptation to stress and other environmental factors, or with alternative means such as pharmacological stimulation of this system.

By and large, this chapter has shown that adaptational protection of the heart is not an exclusive domain of the central stress-limiting systems. An important part in this phenomenon can be played by the local stress-limiting systems forming in the organ itself. Furthermore, the scope of factors involved is most likely not limited to the antioxidant, PG, and adenosinergic systems considered above, but includes the cytoprotective mechanisms operative in the subcellular structures (myofibrillar membranes, mitochondria) and even the DNA genetic template. How true is this assertion we shall see in the next chapter.

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Chapter 6

THE PHENOMENON OF ADAPTIVE STABILIZATION OF STRUCTURES AND HEART PROTECTION

I. INTRODUCTION

The question in what measure the adaptational protection of the heart is due to changes in neurohormonal regulation and in what measure to changes in the heart muscle cells has remained open until quite recently. A most simple approach to this problem was obviously to elucidate whether or not the cardioprotective effect of adaptation observed in the whole organism is retained when the isolated hearts of adapted animals are exposed to injurious factors. In accordance with this, we have studied the effect of adaptation on the resistance of the isolated heart to ischemia and reperfusion, to high catecholamine and calcium concentrations, and finally its effect on the inactivation of some cardiomyocyte structures during autolysis.

II. EFFECT OF ADAPTATION TO REPEATED STRESS ON ISOLATED HEART RESISTANCE TO ISCHEMIC AND REPERFUSION DAMAGE

Our experiments¹ have shown that a fruitful model for studying the effects of adaptation is offered by ischemic and reperfusion arrhythmias.

The diagram in Figure 1A demonstrates that in a 20-min acute ischemia produced with coronary artery ligation, the total duration of grave arrhythmias (ventricular tachycardia and ventricular fibrillation) is the greatest in conscious closed-chest animals, 40% less in anesthetized ones ($p < 0.01$), and 25 times less in isolated hearts ($p < 0.001$). At present this is not at all surprising, being quite in line with the concept of the decisive role of cardiac neural regulation disturbances, and first of all of the excessive adrenergic effect on the heart, in the pathogenesis of ischemic arrhythmias.

In other words, we are again facing the obstinate fact that in local ischemic injury to the heart the evolving arrhythmias are of central genesis. Figure 1B shows that the picture is different in reperfusion arrhythmias developing in response to restitution of blood flow through the ischemic myocardium. Here the differences in the arrhythmic response of the heart in the whole organism and in isolation are smaller than and opposite to those in ischemia: the duration of reperfusion arrhythmias in isolated hearts is 60% greater than in conscious animals ($p < 0.05$). This also should not be surprising, since complete prolonged clamping of the coronary artery followed by its abrupt reopening brings on simultaneously the oxygen and the calcium paradoxes.² This gives rise to at least two phenomena that are often fatal for the heart. The first one is marked activation of lipid peroxidation (LPO) and other components of the lipid triad, with massive destruction of the cell membranes and ensuing calcium entry into the cell. The second one is the cardiotoxic effect of calcium: myofibrillar contracture, uncoupling of oxidative phosphorylation in mitochondria, activation of phospholipases and proteases, and breakdown of cell structures. In the whole organism these essentially local effects can be to some extent additionally checked by the extracardiac antioxidant and calcium-binding agents, whereas in the isolated heart there are only local cell mechanisms to oppose them. As a result, both the injury as such and its arrhythmogenic effect turn out to be greater.

These data prompt a conclusion that *the ischemic arrhythmias are elicited mainly through the central regulatory mechanisms, while the reperfusion ones through the local cardiac*

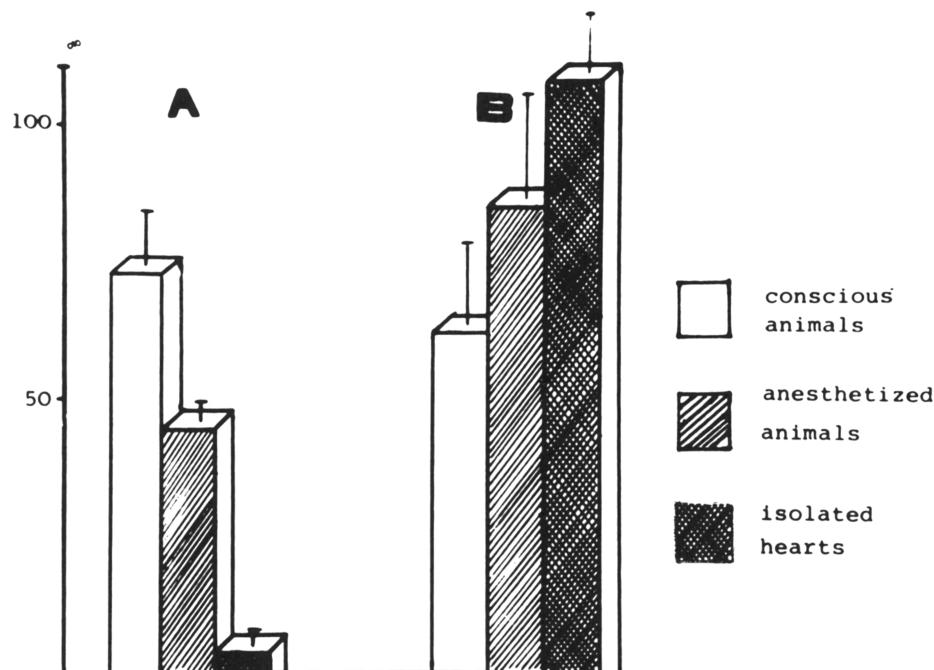


FIGURE 1. Overall duration of (A) ischemic and (B) reperfusion arrhythmias (ventricular tachycardia plus ventricular fibrillation) in conscious and anesthetized animals and in isolated hearts.

mechanisms, and consequently in the adaptational self-defense of the heart the ischemic arrhythmias can be attenuated or abolished mainly owing to activation of the central stress-limiting systems, while the reperfusion arrhythmias mainly by activation of the local systems.

To assess the validity of this assertion, we have conducted experiments to elucidate how the protective antiarrhythmic effect of adaptation to stress is realized in the ischemic and reperfused isolated heart, i.e., under conditions where the central stress-limiting systems can certainly be of no appreciable importance.

Rat hearts were isolated according to Langendorff; ischemia was produced by ligating the left descending coronary artery for 20 min, then the ligature was loosened and the heart monitored in reperfusion for 10 min. The main results are presented in Table 1.

One can see that through 20 min of ischemia all control and adapted hearts retained their contractile activity, but the total duration of arrhythmias in adapted hearts was 36% less than in controls; the ischemic arrhythmias were represented mostly by extrasystoles.

Reperfusion elicited much more grave arrhythmias in both groups, and the protective effect of adaptation proved much more pronounced. Thus, the total duration of arrhythmias in the adapted hearts decreased almost by half. The most serious form — fibrillation — developed in 8 out of 10 control hearts, but only in four adapted ones. The total duration of fibrillation in the adapted group was smaller than in the control group by a factor of 7.3. Finally, seven adapted hearts, but only three control hearts, were still working by the 10th min of reperfusion.

The general issue is that *in the course of adaptation to short stress exposures a local mechanism is established in the heart that efficiently protects it from reperfusion arrhythmias and is less effective against the ischemic ones.*

In further studies, it was important to find out whether or not this local antiarrhythmic mechanism can have direct cytoprotective action, i.e., whether it protects the myocardial cells from reperfusion injury. To this end, we made use of the "reperfusion paradox"

TABLE 1
Effect of Prior Adaptation to Stress on Ischemic and
Reperfusion Arrhythmias in Isolated Hearts

Indices	Acute ischemia		Reperfusion	
	Control (n = 10)	Adapted (n = 10)	Control (n = 10)	Adapted (n = 10)
Extrasystole				
Number of hearts	7	6	10	10
Total duration (s)	215	129	753	1079
Ventricular tachycardia				
Number of hearts	6	4	10	10
Total duration (s)	22	24	1317	1374
Mean duration	2.2 ± 1.1	2.4 ± 1.1	132 ± 42	137 ± 67
Ventricular fibrillation				
Number of hearts	0	0	8	4
Total duration (s)	0	0	3198	440
Mean duration	0	0	319 ± 55	44 ± 42*
All arrhythmias				
Number of hearts	7	6	10	10
Total duration (s)	273	153	5268	2893
Mean duration	27 ± 9	15 ± 9	526 ± 26	289 ± 69 ^b
Number of hearts arrested	0	0	7	3

* $p < 0.05$.

^b $p < 0.01$.

discovered by Hearse et al.² Figure 2 shows the response of isolated control and adapted hearts to reperfusion following a 15-min total ischemia. Adaptation can be seen to largely check the depression of contraction amplitude and to decrease the extent of contracture in the reperfusion paradox. Of especial interest are the curves in part C, which reflect the leakage of creatine phosphokinase (CPK) into the perfusate owing to sarcolemmal damage. The enzyme leakage and hence the membrane damage are much less in the adapted hearts. Thus, there is unambiguous evidence that adaptation of the whole organism to stress results in formation in the heart proper of some mechanism that protects the cardiac cells (in particular, their sarcolemmal membrane) from reoxygenation damage.

III. LOCAL ADAPTATIONAL PROTECTION OF THE HEART AGAINST TOXIC CATECHOLAMINE LEVELS AND EXCESS CALCIUM

After ischemia and reperfusion, the next important factor damaging the heart in natural conditions is the excess of catecholamines and the catecholamine-induced excessive entry of calcium into the sarcoplasm. Accordingly, our experiments were aimed at evaluating the resistance of isolated adapted hearts to both of these.

When the isolated heart is perfused with toxic doses of epinephrine ($5 \times 10^{-5} M$), there evolves sinus tachycardia with marked impairment of contractility (a severalfold decrease in contraction amplitude and contraction and relaxation velocities, and large contracture) as well as rhythm disorders such as numerous extrasystoles and atrioventricular (AV) block (Figure 3). Prior adaptation to stress largely attenuates the epinephrine-induced arrhythmias, as well as the adrenotoxic contracture and impairment of cardiac contractility. In this context, it is essential that the adrenergic heart injury is in great measure due to cAMP-mediated activation of the slow Ca^{2+} channels through which excess calcium enters the cardiomyocytes. Therefore, detailed studies were then carried out on the resistance of isolated control and adapted hearts to contractual and arrhythmogenic effects of high calcium.

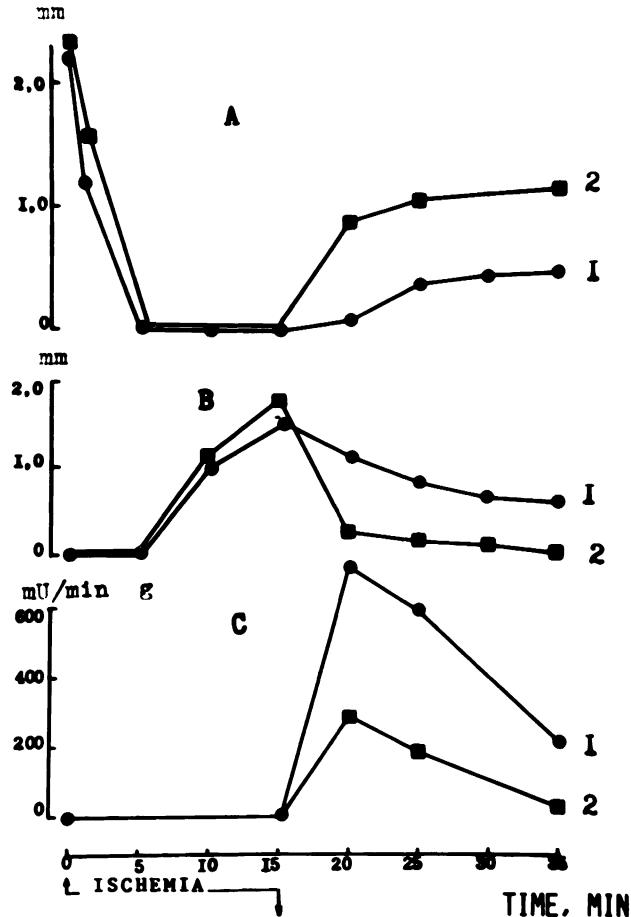


FIGURE 2. Effect of adaptation to stress on (A) contraction amplitude, (B) extent of contracture, and (C) leakage of CPK from isolated rat hearts in ischemia and reperfusion. (1) Control, (2) adapted.

With isotonically contracting hearts from control and adapted animals, the calcium concentration in the perfusing solution was raised from normal 1.36 to 10 mM. Changes in pH and osmolarity were insignificant and therefore not compensated for. The hypercalcium perfusion was carried out for 2 min. The contracture was determined by relating the diastolic apicobasal length of the heart to its length at rest before the calcium shift-up. Three types of ventricular arrhythmias — extrasystole, tachycardia, and fibrillation — were characterized by (1) the number of hearts with each type; (2) the total duration of the given type in the whole group; and (3) the mean duration of the given type per one heart in the group (taking into calculation the total number of hearts in the group regardless of the occurrence of arrhythmias).

The recordings in Figure 4 allow a qualitative assessment of the action of excess calcium on the control and the adapted hearts. The ECGs show that ventricular extrasystoles appear in 15 to 20 s of high calcium in the control heart, but not in the adapted one. As to isotonic contractility, the calcium shift-up first produced a positive inotropic effect in both groups, then in the control the diastolic relaxation was impaired, giving rise to large transient contracture and a pronounced decrease in the contraction amplitude, which were both negligible in the adapted hearts.

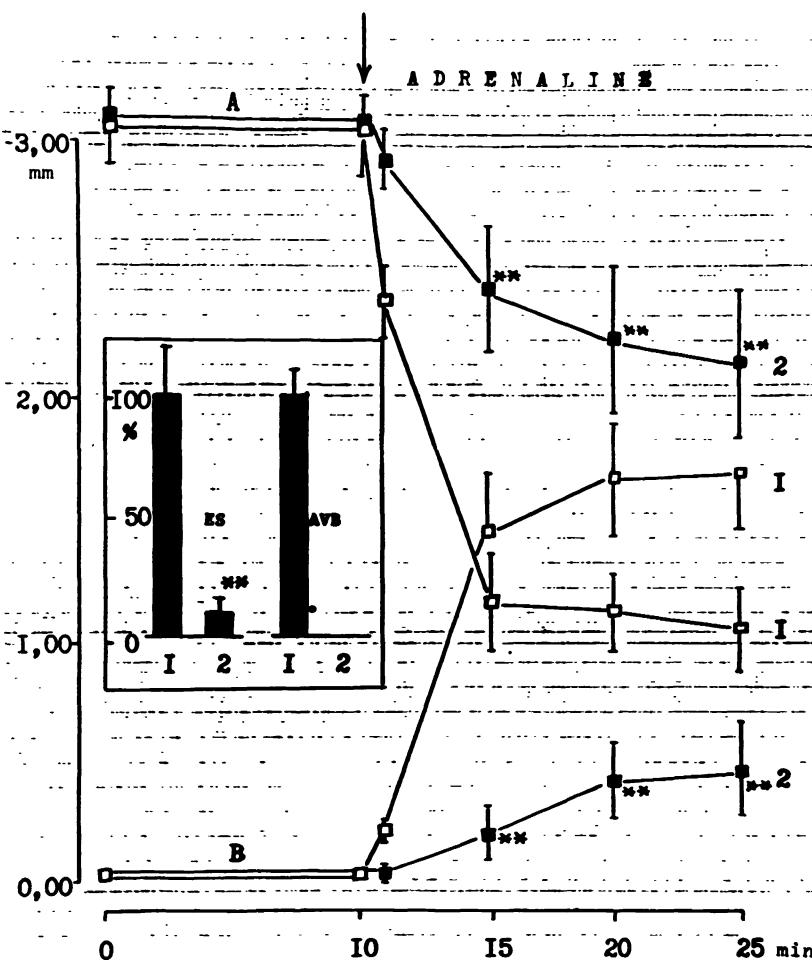


FIGURE 3. Effect of adaptation to stress on (A) contraction amplitude, (B) contracture, and (inset) arrhythmias in isolated hearts exposed to cariotoxic doses of epinephrine ($5 \times 10^{-5} M$). (1) Control, (2) adapted. (ES) Extrasystole, (AVB) atrioventricular block.

The quantitative evaluation of the antiarrhythmic and anticontractural effect of adaptation can be found in Table 2. At first, in both groups the excess of calcium increases the contraction amplitude by 30%, but then the amplitude declines in the control, but remains high in the adapted hearts, where the contracture proves to be three times less. The number of extrasystoles in adaptation is fivefold lower than in the control.

Thus, *prior adaptation of animals to short, noninjurious exposures to stress markedly attenuates the contractural and arrhythmogenic action of excess calcium on the isolated heart.*

At the next stage of our work, calcium was removed from the perfusing solution for 5 min and then returned to normal. As shown in Figure 5, removal of calcium disrupts the electromechanical coupling in both control and adapted hearts; as it does so, in adaptation the AV block develops after a longer latent period (222 ± 30 s vs. 132 ± 24 s in the control, $p < 0.05$). The major differences between the two groups are seen as the calcium concentration is restituted, and the control hearts develop contracture attended with decreased contraction amplitude and pronounced group extrasystolia, whereas the adapted hearts display practically such phenomena.

TABLE 2
Effect of Adaptation on the Contractility and Rhythm Disorders of Isolated Rat Hearts in Exposure to High Calcium Concentrations

Indices	Calcium conc. (mM)	
	1.36	10
Maximal contraction amplitude (mm)		
Control (n = 7)	2.78 ± 0.21	3.59 ± 0.21
Adapted (n = 7)	2.56 ± 0.17	3.42 ± 0.20
Minimal contraction amplitude (mm)		
Control		2.54 ± 0.19
Adapted		3.30 ± 0.18*
Contracture (mm)		
Control	0	1.04 ± 0.19
Adapted	0	0.36 ± 0.09 ^b
Contraction rate (beats per minute)		
Control	277 ± 10	291 ± 14
Adapted	270 ± 7	313 ± 15
Coronary flow (ml/min)		
Control	9.6 ± 0.7	13.7 ± 1.0
Adapted	8.7 ± 0.6	13.5 ± 0.7
Total number of extrasystoles		
Control	0	76
Adapted	0	13
Mean number of extrasystoles		
Control		11 ± 4
Adapted		2 ± 1*

* p < 0.05.

^b p < 0.01.

The anticontractural and the antiarrhythmic effects of adaptation are quantitated in Tables 3 and 4. One can see that at the same spontaneous contraction rate and coronary flow after returning calcium to the perfusing solution, in adapted hearts the contracture is 2.5 times less and, conversely, the contraction amplitude is twice higher than in the controls (Table 3). Concurrently, within the first 5 min after calcium restitution the number of ventricular extrasystoles per heart in the adapted group is about half that in the control, the incidence of ventricular tachycardia is 2.5 times less, and its mean duration is over 14 times less (Table 4).

In the aggregate these data provide convincing evidence for the greater capacity of the membrane mechanisms of calcium homeostasis in the adapted hearts with regard to abolishing such detrimental effects of excess calcium as contracture and arrhythmia. This in all probability is achieved through faster removal of Ca^{2+} from the sarcoplasm. There are three main mechanisms responsible for this process, namely the sarcoplasmic reticulum (SR) calcium pump, the sarcolemmal calcium pump, and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger.

To assess the probable role of each of these mechanisms in the protective effect of adaptation, at the next stage of our work we loaded the papillary muscle cells with calcium using a low-sodium solution. Rat right ventricular papillary muscle was placed into a temperature-controlled (30°C) chamber with carbogen-saturated Krebs-Henseleit solution at pH 7.3 to 7.4. The perfusion rate was 10 to 12 ml/min. One end of the muscle was fixed rigidly and the other was connected to a TD-112 isotonic sensor. The experiment was preceded by a 1-h stabilization period when the muscle was stimulated with rectangular 5-m electric impulses with an amplitude equal to 1.5 to 2 threshold values at a frequency of 0.5 Hz.

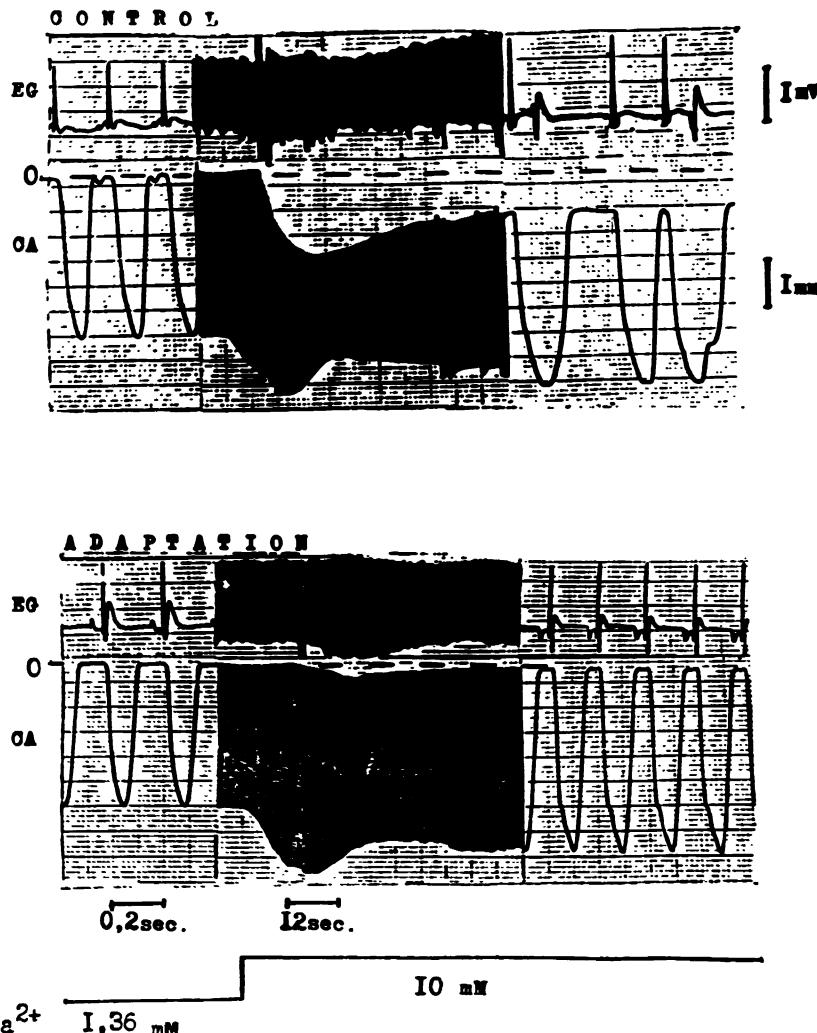


FIGURE 4. Effect of adaptation to stress on the electric and mechanical activity of the isolated rat heart at high Ca^{2+} concentration. (EG) Electrogram, (CA) isotonic contraction amplitude. For explanations see text.

The stimulation was carried out with silver chloride electrodes and a SEN 3201 stimulator (Nihon Kohden, Japan). The recording equipment was the same as in previous works.

The bioelectric activity of myocardial cells was measured with the use of "floating" microelectrodes made from glass capillaries filled with 3 M KCl and connected via a 30 μm tungsten wire to a Nihon Kohden MEZ 8201 preamplifier, wherefrom the signal was supplied to an oscilloscope and/or into the memory of Nihon Kohden RAT 1100 for subsequent analysis.

Before the experiment the muscle was stretched to a length providing the maximal contraction amplitude. The muscle was stimulated for 2 min at a frequency of 3 Hz, then stimulation was discontinued and the normal solution was replaced with the low-sodium one (9 mM sodium with sucrose as a substitute); the development of hyponatremia contracture was monitored for 10 min with recording of the rest potential (RP).

In such an experiment, calcium enters the cell through the $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism and accumulates in the sarcoplasm to a very high concentration; this cannot be

TABLE 3
**Effect of Adaptation on Isolated Rat Heart Contractility in Restitution
of Normal Calcium after Calcium-Free Perfusion**

Indices	Calcium conc. (mM)		
	1.36	0, 5 min	1.36, 5 min
Contraction amplitude (mm)			
Control (n = 7)	2.63 ± 0.18	0	0.73 ± 0.18
Adapted (n = 7)	2.50 ± 0.19	0	1.53 ± 0.30*
Contracture (mm)			
Control	0	0.58 ± 0.04	1.09 ± 0.09
Adapted	0	0.41 ± 0.10	0.44 ± 0.19*
Contraction rate (beats per minute)			
Control	277 ± 12	AV block	237 ± 20
Adapted	269 ± 10	AV block	240 ± 7
Coronary flow (ml/min)			
Control	9.5 ± 0.6	13.5 ± 0.7	8.5 ± 0.8
Adapted	8.3 ± 0.6	13.4 ± 0.7	7.6 ± 0.6

* p < 0.05.

TABLE 4
**Effect of Adaptation on Arrhythmias in Isolated Hearts Caused
by Restitution of Normal Calcium after Calcium-Free Perfusion**

Indices	Control (n = 7)	Adapted (n = 7)
Extrasystole		
Number of hearts	7	7
Total number of extrasystoles	315	175
Mean number of extrasystoles per heart	45 ± 7	25 ± 5*
Ventricular tachycardia		
Number of hearts	5	2
Total duration (s)	41	3
Mean duration	5.8 ± 2.0	0.4 ± 0.6*
Ventricular fibrillation		
Number of hearts	2	0
Total duration (s)	18	0

* p < 0.05.

abolished or attenuated by the sarcolemmal Ca ATPase, which is responsive in the low calcium range and normally participates only in the final stage of diastole.³ Hence the main burden is to be taken by the SR calcium pump.

Figure 6 shows that in the initial solution the muscles from control and adapted animals contract with approximately the same amplitude (10 to 12% of their diastolic length). The low-sodium solution elicits great differences between them: in the control, within 10 min contracture reaches a plateau, with a stable shortening of the muscle close to its contraction amplitude under stimulation in the Krebs-Henseleit solution; with the adapted muscle the contracture is insignificant.

The quantitative results of the whole series of these experiments are presented in Figure 7: in the control group the contracture in 10 min of low-sodium perfusion approximates the normal contraction amplitude, whereas in the adapted group it is fivefold less. Accordingly, the RP declines to 57 mV in the control, but remains at 68 mV in the adapted group. This is most probably explained by the fact that adaptation enhances the efficiency of the SR calcium pump.

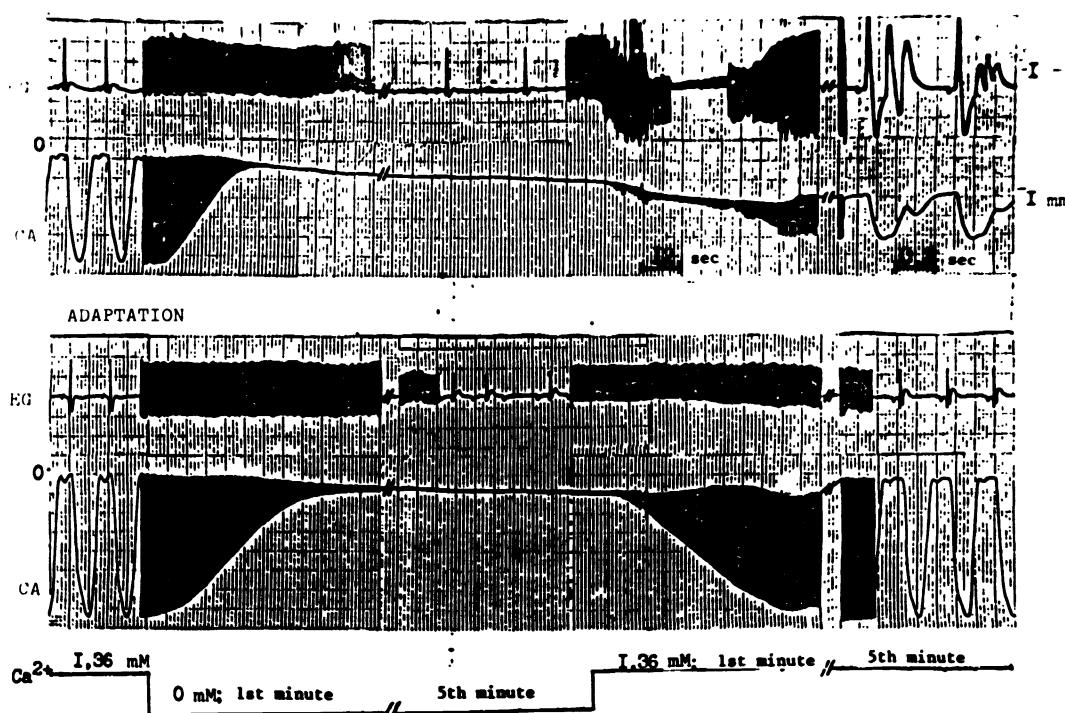


FIGURE 5. Effect of adaptation to stress on the electric and mechanical response of the isolated heart to calcium-free perfusion and restitution of calcium. Designations are as in Figure 4. For explanations see text.

To study further the mechanisms of the antiarrhythmic effect of adaptation, we used the same technique with papillary muscle to evaluate the main electrophysiological parameters of the myocardial cells under an increase of such a physiological determinant of calcium loading as the contraction frequency at normal or elevated calcium.

There are several issues from Table 5. First, at increasing contraction rate and high calcium the RP is reliably lower in control than in adaptation (69 mV vs. 82 mV), i.e., the difference is qualitatively the same as with low sodium. Since in combined action of high calcium and imposed contraction rate the calcium load is mainly created owing to its entry through the sarcolemma, this is real evidence for the adaptively enhanced ability of the cardiomyocytes to maintain the RP in calcium overload that may be caused by different mechanisms.

Second, owing to the normal RP retained at maximal calcium load (high calcium and 3 Hz) the amplitude of the action potential (AP) in adapted specimens exceeds the control one by 25%, which should favor the maintenance of a high enough conduction velocity under such conditions.

Third, the most consistent observation is that the well-known phenomenon of shortening RP with increasing contraction rate at physiological and high calcium levels is much less pronounced in the papillary muscle from adapted animals. As a result, at 180 contractions per minute the duration of the AP was twice longer in the adapted group at 10 to 90% repolarization. Thus, prior adaptation to repeated stress undoubtedly prolongs the AP and hence the effective refractory period at near-physiological contraction rates; this change by itself must diminish the probability of premature depolarization.

The data on papillary muscle contractility obtained in the same experiments are presented in Table 6 and show that at physiological calcium concentrations accelerated contractions

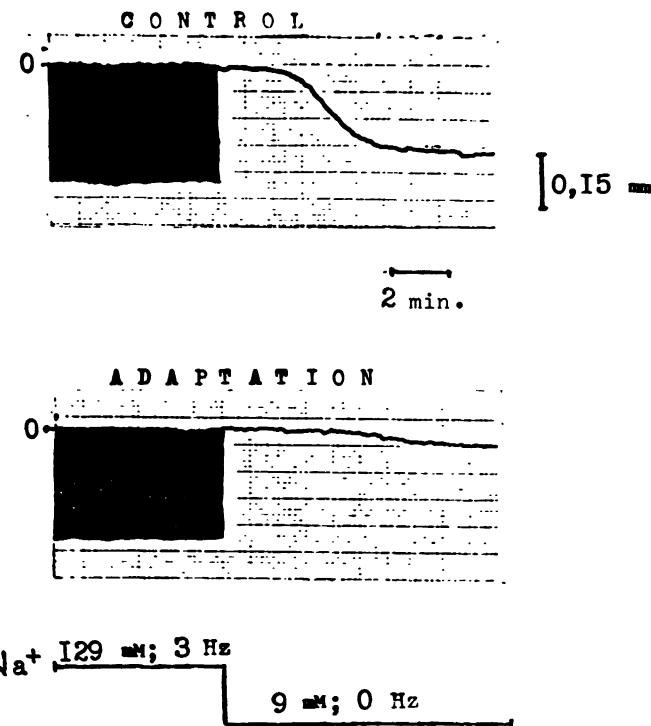


FIGURE 6. Effect of adaptation to stress on the hyponatrium contracture of papillary muscle. The tracings present the changes in muscle length; the "0" level corresponds to complete diastolic relaxation.

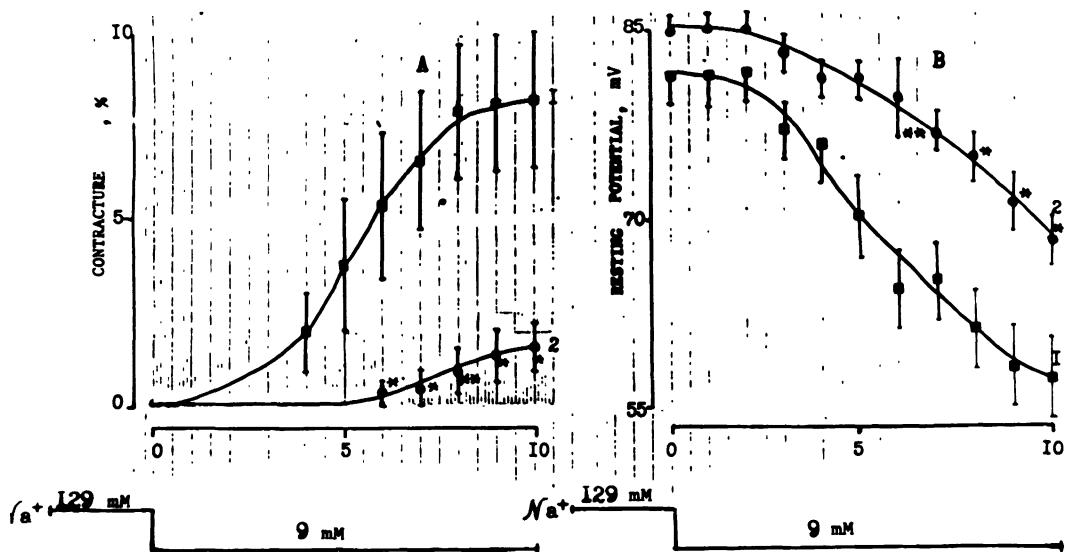


FIGURE 7. Effect of adaptation to stress on (A) contracture and (B) rest potential of the rat papillary muscle exposed to low-sodium solution (9 mM Na⁺).

TABLE 5
Effect of Adaptation on the Bioelectric Activity of Papillary Muscle Cells

Indices	2.5 mM Ca ²⁺ Pacing Frequency (Hz)			18 mM Ca ²⁺ Pacing frequency (Hz)		
	0.5	1.0	3.0	1.0	3.0, 5 min	3.0, 10 min
Overshoot (mV)						
Control	28.3 ± 1.6	30.0 ± 3.8	14.1 ± 1.9	33.0 ± 3.0	4.3 ± 5.6	5.4 ± 5.8
Adapted	23.4 ± 3.4	21.0 ± 3.2	13.1 ± 2.4	27.7 ± 3.9	13.0 ± 4.4*	18.0 ± 5.1*
Rest potential (mV)						
Control	75.3 ± 2.9	77.5 ± 3.0	69.0 ± 1.2	87.5 ± 2.0	75.8 ± 3.0	69.0 ± 5.0
Adapted	79.8 ± 1.1	76.8 ± 2.2	74.3 ± 2.7	83.8 ± 2.8	75.4 ± 3.4	82.0 ± 3.0*
Action potential (mV)						
Control	104.4 ± 1.8	107.5 ± 3.4	83.2 ± 4.3	120.3 ± 1.8	80.6 ± 6.6	74.0 ± 8.9
Adapted	103.3 ± 3.5	97.8 ± 3.2	87.4 ± 3.1	111.0 ± 2.4*	88.0 ± 4.0	99.8 ± 4.0*
Duration of repolarization (m)						
10%	Control	6.0 ± 0.8	5.0 ± 0.7	3.5 ± 0.7	6.2 ± 0.8	1.6 ± 0.2
	Adapted	7.6 ± 0.6	7.0 ± 0.7	6.2 ± 0.8*	6.7 ± 0.6	3.7 ± 0.8*
30%	Control	15.0 ± 1.6	14.0 ± 1.9	6.2 ± 1.0	11.0 ± 1.0	3.2 ± 0.3
	Adapted	16.5 ± 1.1	15.7 ± 1.4	12.0 ± 1.5*	12.7 ± 0.8	6.2 ± 1.4*
50%	Control	25.8 ± 3.0	23.4 ± 3.3	9.8 ± 1.5	16.3 ± 1.4	5.2 ± 0.6
	Adapted	29.3 ± 2.1	30.2 ± 2.7	18.3 ± 2.4*	18.9 ± 0.7	8.8 ± 1.9
70%	Control	81.4 ± 16.0	63.0 ± 12.0	17.7 ± 3.7	29.5 ± 2.9	8.1 ± 0.9
	Adapted	110.0 ± 16.9	98.0 ± 11.0	38.1 ± 7.9*	47.8 ± 4.0*	13.8 ± 3.0
90%	Control	215.0 ± 16.0	196.0 ± 22.0	50.0 ± 13.0	107.0 ± 24.0	22.1 ± 5.2
	Adapted	235.0 ± 13.0	220.0 ± 7.0	106.4 ± 18.5*	185.0 ± 12.7*	35.0 ± 5.1

* p < 0.05.
 b p < 0.01.

TABLE 6
Effect of Adaptation on the Contractility Parameters of Rat Papillary Muscle

Indices	2.5 mM Ca ²⁺ Pacing frequency (Hz)			18 mM Ca ²⁺ Pacing frequency (Hz)		
	0.5	1.0	3.0	1.0	3.0, 5 min	3.0, 10 min
Contraction amplitude (% initial muscle length)						
Control (n = 7)	21.8 ± 1.2	20.8 ± 1.4	11.0 ± 2.0	18.0 ± 1.4	4.3 ± 1.1	1.1 ± 0.1
Adapted (n = 7)	19.5 ± 1.0	18.9 ± 0.8	12.8 ± 1.2	17.8 ± 0.9	10.2 ± 2.2 ^a	4.7 ± 1.2 ^a
Contraction rate (muscle length units/s)						
Control	1.46 ± 0.07	1.43 ± 0.06	0.77 ± 0.14	1.06 ± 0.10	0.28 ± 0.08	0.09 ± 0.01
Adapted	1.27 ± 0.07	1.27 ± 0.07	0.93 ± 0.04	1.13 ± 0.05	0.66 ± 0.13 ^a	0.35 ± 0.09 ^a
Relaxation rate (muscle length units/s)						
Control	1.14 ± 0.06	1.10 ± 0.09	0.64 ± 0.13	0.79 ± 0.09	0.20 ± 0.05	0.09 ± 0.01
Adapted	0.98 ± 0.05	0.98 ± 0.05	0.74 ± 0.08	0.80 ± 0.07	0.58 ± 0.13 ^a	0.32 ± 0.08 ^a
Contracture (% initial muscle length)						
Control	0	0	0	1.5 ± 1.5	15.4 ± 1.3	28.4 ± 2.1
Adapted	0	0	0	0	4.6 ± 2.3 ^b	12.5 ± 2.8 ^c
Contracture (% initial contraction amplitude)						
Control	0	0	0	13	140	258
Adapted	0	0	0	0	36 ^a	97 ^b

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

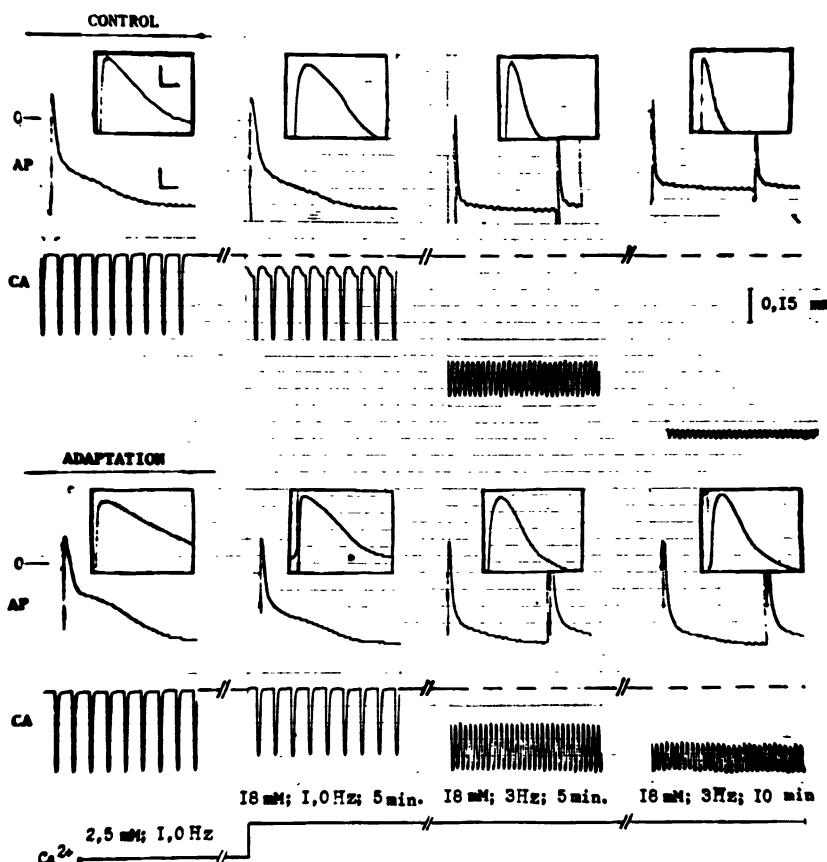


FIGURE 8. Effect of adaptation to stress on the electric activity (action potential, AP) and isotonic contraction (contraction amplitude, CA) of the papillary muscle exposed to high calcium and increased pacing frequency. The insets show the AP tops at higher sweep.

result in a negative inotropic effect, with no differences between the two groups. At high calcium and 180 contractions per minute, the adapted muscles exceed the controls twofold to fourfold in the contraction amplitude and contraction and relaxation velocities; this is so because upon adaptation the calcium-induced contracture proves to be 2.5 times less. Thus, in our experiments adaptation was equally effective against the hypercalcium contracture in ventricles and papillary muscles of isolated hearts. This obviously could only be due to enhanced calcium binding and removal.

The data summarized in Tables 5 and 6 are illustrated by experimental recordings in Figure 8. As can be seen, the high calcium load at a stimulation frequency of 180 min^{-1} greatly "narrows" the AP of myocardial cells and almost completely suppresses the contractility of the papillary muscle. In a specimen from adapted animal, the AP duration is much less affected and contractility is retained at the same calcium load.

Thus, on the one hand we have earlier shown that adaptation to stress is effective against adrenergic, ischemic, and reperfusion arrhythmias, and on the other, here we see that it has antiarrhythmic and anticontractural effects against calcium overloading of the cardiac muscle, whatever its cause. This is not likely to be just a coincidence, and is most probably due to the fact that an excess of calcium accumulates in the cardiac cells and acts arrhythmogenically in all types of heart damage against which adaptation has proved effective (acute ischemia

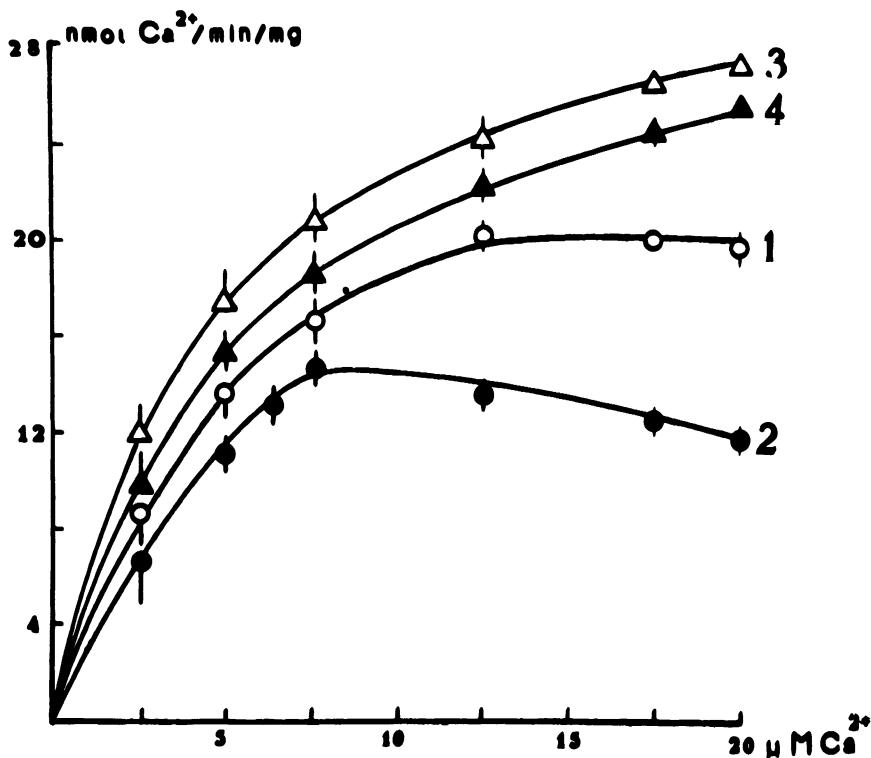


FIGURE 9. Dependence of the calcium uptake rate by the SR on Ca^{2+} concentration in the medium.
 (1) Control, (2) single prolonged stress, (3) adaptation to stress, (4) adaptation plus prolonged stress.

and reperfusion, acute myocardial infarction, emotional pain stress [EPS]). Indeed, increased calcium entry into the cell or its excess in the sarcoplasm have been demonstrated in ischemia and reperfusion,⁴ and in EPS⁵ attended with a drop in the fibrillation threshold. In all such cases enhanced calcium-removing capacity would diminish the probability of arrhythmias. Our data on the direct protective effect of adaptation against excess calcium provide unequivocal support to this suggestion.

Along this line, we next assessed the effect of adaptation on the capacity of the calcium-transporting mechanisms, and on the resistance of these and other cell components to autolysis, which is a major necrobiotic factor.

IV. EFFECT OF ADAPTATION TO STRESS ON THE FUNCTION OF THE SARCOPLASMIC RETICULUM CALCIUM PUMP AND MITOCHONDRIA AND ON THEIR RESISTANCE TO AUTOLYSIS

The studies on the SR calcium pump⁷ included four series of experiments on rats: (1) control, (2) prolonged (6 h) immobilization stress, (3) adaptation to short (1 h) stresses, and (4) prolonged immobilization stress on the next day after completion of adaptation. Rats were sacrificed by decapitation 2 h after stress or 1 d after the end of adaptation. Hearts were extracted, washed with saline, and frozen in liquid nitrogen. Homogenates were prepared and Ca^{2+} uptake by the SR vesicles was measured ionometrically as described in Chapter 1, Section VIII.

Figure 9 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

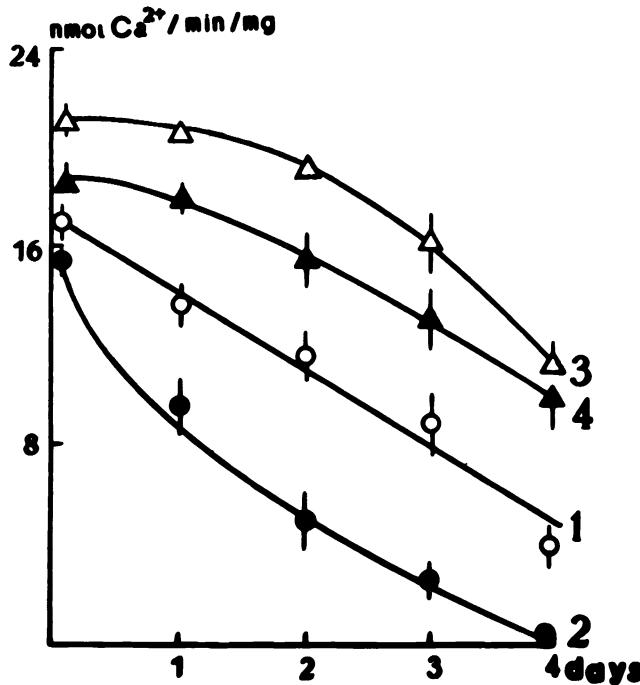


FIGURE 10. Decline in the rate of calcium uptake by rat myocardial SR vesicles during storage of the homogenates at 4°C. (1) Control, (2) single prolonged stress, (3) adaptation to stress, (4) adaptation plus prolonged stress.

concentration, reaching a plateau at 1 to $2 \cdot 10^{-5} M$ Ca²⁺. Upon prolonged stress the uptake is slower and its rate reaches a maximum already at 7.5 μM ; moreover, at higher calcium concentrations it is inhibited so that at 20 μM it is almost twice lower than in the control.

It can further be seen that adaptation to short stresses results in substantial enhancement of calcium uptake (e.g., a 30% surplus at 5 μM Ca²⁺), and the uptake rate still increases at high calcium concentrations without reaching a plateau. The same pattern is observed in stress after adaptation, so that after prolonged stress the uptake rate in adapted animals is reliably higher than in nonadapted ones at both physiological and elevated calcium concentrations. Thus, prior adaptation not only itself enhances the calcium-accumulating ability of cardiac-cell SR, but certainly protects the SR membranes from the adverse influence of prolonged immobilization stress.

It is known that the state of the SR calcium pump depends in great measure on its lipid microenvironment which is exposed to such endogenous cell factors as lipases, phospholipases, LPO, etc. Since the activity of such factors has been proved to change in stress and adaptation to stress, these changes may affect the SR calcium transport. Therefore, we have studied the dynamics of inactivation of the SR calcium pump during storage of homogenates, whereby the cell structures have been exposed to endogenous degrading factors.

Figure 10 shows the decline in the calcium uptake rate in SR as the preparations are kept at 4°C. In the control this process is linear and about 20% of initial activity is retained in 4 d. After stress the inactivation is much faster and in 4 d no uptake is observable. Adaptation, besides enhancing the initial uptake rate, greatly retards its decline; even after stress following adaptation the SR retains half of its activity in 4 d. Thus, adaptation makes the SR membrane so much more resistant to degrading factors that, even upon stress, its calcium pump remains markedly more stable than in the control.

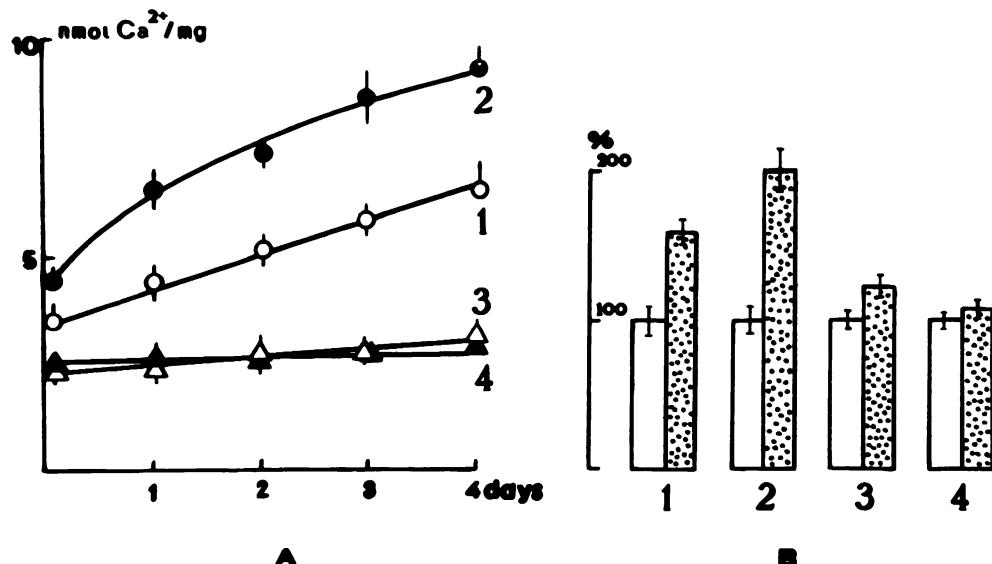


FIGURE 11. Release of calcium in myocardial homogenates during storage at 4°C. (A) Free Ca^{2+} content at tissue to medium ratio of 1:4. (B) Relative increase in free calcium content in 3 d. (1) Control, (2) single prolonged stress, (3) adaptation to stress, (4) adaptation plus prolonged stress.

Another criterion for assessing the state of myocardial membrane structures can be the dynamics of accumulation in the homogenates of free calcium released from the SR and mitochondria. As shown in Figure 11, such leakage is clearly observable in the control and still more pronounced upon stress; after adaptation, the release of free calcium is greatly diminished, and upon stress following adaptation there are no appreciable changes throughout the 4 d of incubation. *This striking fact of practically no leakage of calcium from the intracellular depots in the myocardium of animals exposed to stress after prior adaptation testifies to a radically enhanced stability of membrane structures due to adaptation, which can thus block the action of endogenous injuring factors.*

The stress-induced impairment of the SR calcium pump and its faster inactivation in storage appear to be associated, since much the same natural degrading factors are involved in stress damage and autolysis. Indeed, stress is attended with activation of phospholipases⁸ and LPO,⁹ lysosomal labilization and release of proteases,¹⁰ and ensuing damage to membranes and their enzymes — Na,K ATPase¹¹ and SR Ca ATPase.¹² Activation of LPO,^{13,14} phospholipases¹⁵⁻¹⁷ and proteases,¹⁸ lysosomal labilization,¹⁹ and decreasing activity of membrane-bound enzymes^{20,21} have also been shown in autolysis. It is therefore understandable that the stress damage not only depresses the function of the SR calcium pump, but also precipitates its inactivation in autolysis. It is essential that in our experiment stress increased the free calcium content in the homogenate and aggravated its leakage in autolysis. This is quite in line with the *in vivo* accumulation of calcium in the cardiomyocytes damaged by stress or ischemia. Since Ca^{2+} can activate phospholipases and at certain concentrations also LPO, this may complete a “vicious circle” in groups of cells, in which the natural degrading factors increase the leakage of Ca^{2+} from the intracellular depots and the free Ca^{2+} in its turn potentiates their cytotoxic action. In prolonged stress this corresponds to formation of micronecroses and then focal cardiosclerosis (see Chapter 1).

The ability of adaptation to short stress exposures to prevent the impairment of cardiac structure, function, and electric stability in stress-induced, ischemic, and reperfusion damage has been found in 1985 and confirmed in further works.^{22,23} Now it has for the first time

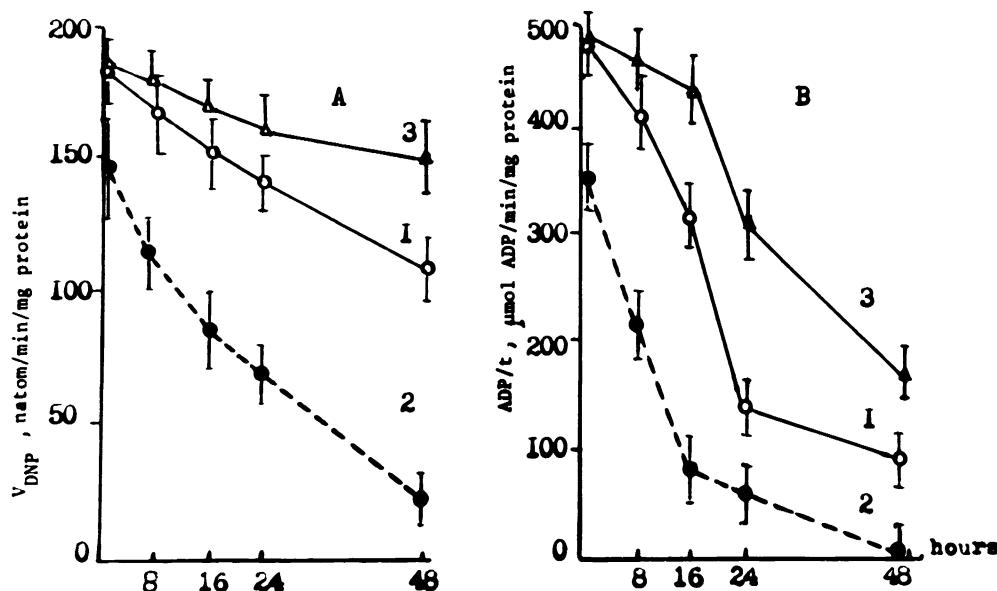


FIGURE 12. Effect of stress and adaptation on the changes in mitochondrial respiration and phosphorylation during storage. (A) Dinitrophenol-uncoupled respiration, (B) phosphorylation rate with succinate as substrate. (1) Control, (2) stress, (3) adaptation.

been elucidated that an important component of the adaptational protection of the heart at the cell level is the enhanced efficiency of the SR calcium pump and the greater ability of the SR membrane to withstand the action of degrading factors.

The fact that adaptation to repeated stress protects the heart from calcium-induced damage at physiological and biochemical levels is in our opinion of principal importance. Indeed, on the one hand calcium is a universal activator of the organismic physiological functions, and on the other it is now well known that excess calcium is the central link of most different types of heart damage. This link is blocked by the mechanisms evoked in adaptation to repeated stress. Hence, development of kinds of adaptation to stress suitable for humans, or isolation and pharmaceutical use of metabolites formed in adaptation, hold much promise for heart protection in a wide range of diseases.

In an attempt to unravel the mechanisms involved in such cardioprotective stabilization, it was first of all necessary to ascertain whether we were dealing with a peculiar phenomenon only specific for the SR membranes or with a general pattern encompassing all cellular structures. In a step along this line, we studied the inactivation of respiration and phosphorylation in mitochondria during prolonged storage and the effect of stress and adaptation on this process, by measuring the generally accepted parameters of respiration and phosphorylation after various times (up to 48 h) of incubation of mitochondrial suspensions at 4°C.

Figure 12A demonstrates the decline in the maximal rates of oxygen consumption upon complete uncoupling with dinitrophenol. As can be seen, in the mitochondria from animals exposed to EPS this index is 15% lower immediately upon isolation and further decreases to a much greater extent. Inactivation of mitochondria from adapted animals is much slower than in the control. Qualitatively, the same pattern is observed with the phosphorylation rate as shown in Figure 12B.

Thus, upon adaptation to repeated stress the ability of mitochondria to withstand natural degradation during autolysis is as certainly enhanced as that of the SR membranes. The experiments considered earlier on the leakage of CPK in reperfusion make it likely that the same stabilization takes place in the sarcolemma.

On the whole, the data available strongly imply that *in the course of adaptation to stress a mechanism is formed in the heart that ensures stabilization of sarcolemma, SR, and mitochondria, i.e., in essence of the main structures of the cardiomyocyte.*

V. ADAPTATIONAL STABILIZATION OF STRUCTURES; MECHANISM AND SIGNIFICANCE FOR HEART PROTECTION

In a wider assessment of the phenomenon of adaptational stabilization of structures, it has been noted that this can take place not only in the heart, but in other organs as well. Furthermore, adaptation to repeated stress appears to be not the only process that can give rise to this phenomenon. Indeed, already in the early 1970s Zak et al.,²⁴ Aschenbrenner et al.,²⁵ and Meerson et al.²⁶ have shown that during such an adaptive reaction as compensatory heart hyperfunction already in the emergency stage there is an increase in the life span of myofibrillar proteins and respiratory chain enzymes. Quite recently it has been found that hyperfunction produces such a stabilizing effect not only on the cell organelles, but also on the DNA template.²⁷ These studies have been considered in detail in Chapter 1, Section V. Here it would suffice to say again that such stabilization is a real phenomenon apparently achieved through local mechanisms.

It is known that in adaptive reactions, and in particular during repeated stress, in the heart there is an increase in the activity of antioxidant enzymes^{28,29} and in the number of adenosine receptors,³⁰ and the membrane phospholipid composition changes towards a lower content of unsaturated fatty acids.³¹ Maybe all these factors somehow contribute to the adaptational stabilization of structures. However, the most intriguing interpretation of this phenomenon emerges if we combine the concept of the regulatory system “inositol triphosphate-diacylglycerol” (IP_3 -DAG) with the concept of so-called heat-shock proteins (HSP).

As applied to our experiments, it can be envisaged (scheme in Figure 13) that reiteration of stress episodes results in reiterated action of stress hormones on the Ca^{2+} -mobilizing receptors, which via phospholipase C activate the IP_3 -DAG regulatory circuit. IP_3 acts on special receptors on the SR to cause release of calcium and is thus the intracellular messenger for immediate adaptive reactions. On the other hand, DAG is a messenger for long-term adaptation: it can act via protein kinase C and the sarcolemmal Na^+/H^+ exchange to activate transcription, cell growth, and proliferation.³⁵ Such activation of the genetic apparatus in hyperfunction and hypertrophy,³⁶ heat exposure,³⁷ hypoxia,³⁶ i.e., in diverse but certainly stressful situations, is attended with accumulation of HSP₇₀, HSP₆₈, and HSP₅₈. These stress proteins reputedly normalize the function of damaged cell nuclei and abolish disturbances of mRNA splicing and protein synthesis.³⁷ The scheme reflects the assumption that in repeated stress the increasing concentration of DAG activates, among others, the genes coding for the stress proteins. These proteins, in their turn, stabilize the structures both at the organellar and the genetic template levels.

In testing the suggestion that stress proteins are involved in the adaptational stabilization of structures, account should be taken of the recent data that the thermal stability of tissues from animals preadapted to heat is proportional to the accumulation of HSP in these tissues.^{38,39} On the strength of these data, we decided to evaluate the heat tolerance of isolated hearts from stress-adapted animals (i.e., the hearts clearly exhibiting the phenomenon of adaptational stabilization). As shown in Figure 14, their thermostability proved to be markedly enhanced. Perfusion with a solution warmed up to 42°C caused severe depression of contractility, pronounced contracture, and massive leakage of CPK in the control hearts, but only slightly affected the adapted ones. This experiment conducted by Malyshev argues in favor of a role of stress proteins in such stabilization. On the whole, however, the hypothesis of the genetic mechanism of the phenomenon of adaptational stabilization of

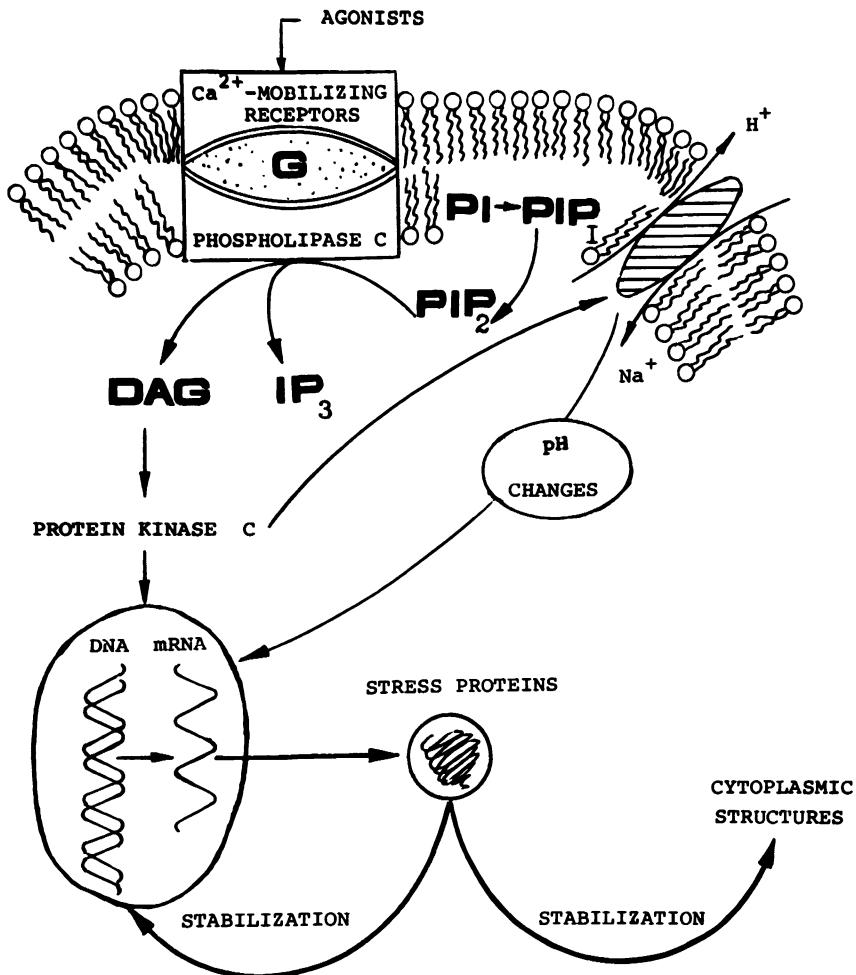


FIGURE 13. A hypothetical mechanism of stabilization of cell structures in repeated stress exposures. (G) G-protein, (PI) phosphoinositol, (PIP) phosphoinositol phosphate, (PIP₂) phosphoinositol diphosphate, (IP₃) inositol triphosphate, (DAG) diacylglycerol.

structures poses many more questions than it does solve: what is the gene-control mechanism underlying the enhanced formation of the stress proteins? do these proteins indeed stabilize the structures? and, finally, is such stabilization achieved through suppressing the natural degrading factors such as phospholipases and proteases or through directly enhancing the resistance of membranes and other cell structures to these factors? Notwithstanding, the phenomenon itself raises no doubt; moreover, it is actually operative, protecting the heart against direct damage from ischemia and ensuring reduction of the infarct size.

This has been demonstrated in a special morphometric study that we carried out together with Shneider and Ustinova. In so doing, we took account of the already classical works of Lowe et al.,⁴⁰ who have shown that despite artery ligation in one strictly definite place, the size of ischemic myocardium may greatly vary with individual animals; at the same time the size of necrosis determined histologically 3 d after artery ligation is directly dependent on and always smaller than the ischemic area, since part of the cells in the periphery of the ischemic region — in the "risk area" — restore their structure and function.⁴¹ As all these zones can be affected by the individual peculiarities of the coronary bed, we thought it

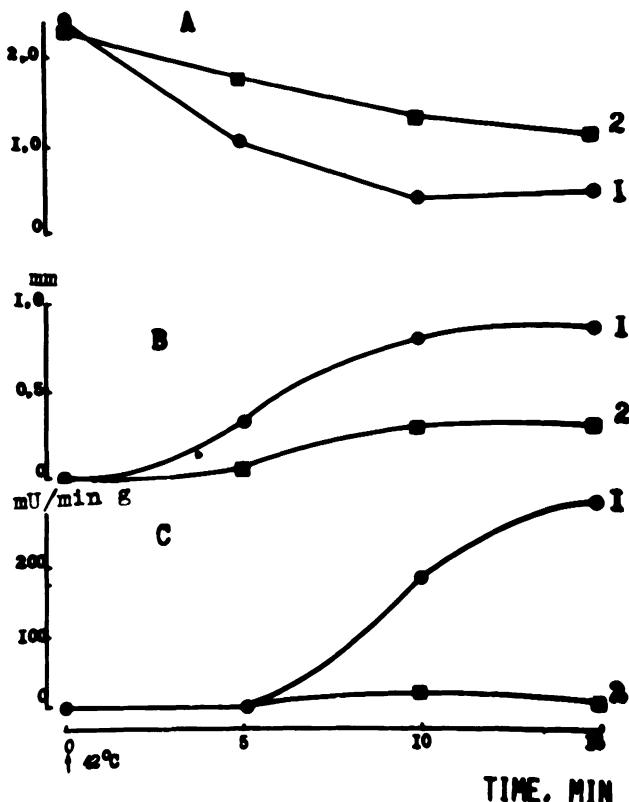


FIGURE 14. Effect of adaptation to stress on (A) contraction amplitude, (B) contracture, and (C) leakage of CPK from isolated hearts exposed to elevated temperature (42°C). (1) Control, (2) adapted.

optimal to measure the ischemic area, the necrotic area, and their difference (the recovery area) in the same animal.

To this end we used a modified method of Lepran et al.⁴² which makes it possible, without opening the chest, to tighten the ligature applied on the coronary artery for 30 min, then to loosen it and 1 d after, when the necrosis has already formed in the myocardium, to tighten it again to reproduce acute ischemia; immediately upon ligation an emulsion of lipid microspheres is rapidly introduced into the left ventricular cavity.⁴³ The heart is then removed, frozen, and sectioned; nonfixed sections are stained with nitroblue tetrazolium to reveal the necrotic area, and sections from formaldehyde-fixed material are stained for lipid to reveal the ischemic area, which is not penetrated by the emulsion. The results are then quantitated into percentages of volume in accordance with the principles of stereological analysis.⁴⁴

Table 7 demonstrates that prior adaptation to stress does not appreciably affect the volume of myocardium that becomes ischemic upon acute coronary artery ligation. At the same time, the adaptation more than doubles the recovery zone in the ischemic myocardium, so that the necrotic volume as related to the ischemic and total myocardial volume decreases nearly by half.

Consequently, adaptation to stress lacks anti-ischemic action, but has a pronounced cytoprotective effect, which can be thought of as a direct manifestation of the phenomenon of the adaptational stabilization of structures. This allows one to think that studies of the genetic mechanisms of this phenomenon are not a purely academic issue; there are two approaches which hold promise for expanding the possibilities of heart protection.

TABLE 7
Effect of Prior Adaptation to Stress on the Ischemic, Recovery, and Necrotic Volumes in Experimental Myocardial Infarction

Series	Percentage			
	Ischemic to total	Necrotic to ischemic	Recovered ischemic to ischemic	Necrotic to total
Control (n = 14)	36.8 ± 3.6	73.5 ± 3.8	26.5 ± 1.7	27.0 ± 1.4
Adapted (n = 11)	34.3 ± 4.1	44.7 ± 4.1	55.3 ± 2.0	15.3 ± 1.4
p		<0.01	<0.01	<0.05

First, it has been found in recent years that a cardioprotective effect in many instances resembling that of adaptation to repeated stress can be experimentally achieved with transauricular acupuncture, in which pulsed currents are passed through subcortical formations of the animal brain to cause moderate catalepsy. In the long run, such courses prevent the impairment of cardiac metabolism and electric stability commonly observed in EPS,⁴⁵ and largely restrict the ischemic and reperfusion arrhythmias.⁴⁶ Recent experiments in our laboratory show that electroacupuncture, like adaptation to stress, brings on myocardial alterations that enhance the direct cardiac resistance to damaging factors. Similar results have been obtained in combined adaptation of animals to moderate intermittent hypoxia and cold. In the aggregate, the data now available suggest that the phenomenon of the adaptational stabilization of structures constitutes the foundation of the currently developing methods for protecting the heart with adaptation.

Second, the stress proteins, some similar factors, or stable synthetic analogs thereof may in future be used to artificially reconstruct this phenomenon (just as the inductor of angiogenesis discovered by Schaper et al.⁴⁷ has now proved capable of being used to cause growth of coronary vessels). In other words, it cannot be excluded that some factors involved in the adaptational stabilization mechanism will find their place among pharmacological means of gene regulation.

The contents of Chapters 3 and 4 have provided unequivocal evidence that the adaptational heart protection is not at all restricted to local cell mechanisms. The next chapter, considering the protection of the heart and of the whole organism with prior adaptation to intermittent hypoxia, offers a good example of efficacious coordination of neurohumoral and cellular factors in the integrated mechanism of long-term adaptation and natural prophylaxis.

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Chapter 7

ADAPTATION TO INTERMITTENT HYPOXIA AND ITS USE FOR PROTECTING THE HEART FROM STRESS AND ISCHEMIC DAMAGE

I. INTRODUCTION

Adaptation to hypoxia follows the general patterns typical of adaptation to any other environmental factor and briefly considered in Chapter 1 (see the scheme in Figure 1 therein). Accordingly, adaptation to hypoxia goes through four main stages. Yet, in the *initial stage* there is some difference, which is first of all due to the fact that unlike the factors causing motor, behavioral reactions, the lack of oxygen initially does not act on extroreceptors, but gradually invades the inner milieu of the organism to cause hypoxemia and to upset thus its homeostasis. Only through hypoxemia the oxygen deficit stimulates the chemoreceptors of the aortal-carotid zone and the centers regulating respiration, circulation, and other organs, eliciting at least three substantial shifts.

First, hypoxemia enhances the function of the systems specifically responsible for oxygen transport from the outer milieu and its distribution in the organism, i.e., causes lung hyperventilation, higher cardiac output, and dilation of brain and cardiac vessels with concomitant narrowing of muscle and abdominal vessels and ensuing rise in blood pressure. Second, there is a fully pronounced stress reaction, in particular a marked catabolic effect, negative nitrogen balance, etc. (see Chapter 1). Third, acute hypoxia directly limits the function of the mitochondrial respiratory chain and thereby the ATP synthesis in various organs and tissues and first of all in the higher divisions of the central nervous system, which entails a more or less pronounced impairment of motor and intellectual activity.

Of course the extent of such shifts may vary depending on the genetically determined individual tolerance of the organism and the depth of hypoxia. Nevertheless, it is just this syndrome attended with large losses of energy and structures that characterizes the initial, emergency stage of adaptation to hypoxia.

In the *second, transitional stage*, the mechanism considered in detail elsewhere¹ activates nucleic acid and protein synthesis in the cells of the systems carrying the main load in adaptation to hypoxia. This results in selective growth of structures that have been limiting oxygen transport and ATP production, i.e., growth of vessels, enhanced erythropoiesis, proliferation of mitochondria, etc.

In the *third stage*, that of stable long-term adaptation, these processes give rise to a complex of structural changes which we have some time ago termed the "systemic structural trace" and which constitutes the material basis of stable long-term adaptation.

In certain cases, e.g., in adaptaion to excessive altitude, hypoxia, or in genetic intolerance to this factor, the process may develop into the *fourth, unnecessary stage* of wear-out when the systemic structural trace is gradually destroyed and adaptation turns into disease.

The aim of this Chapter is to demonstrate, first experimentally and then clinically, that adaptation to moderate, rationally dosed hypoxia can be used to protect the heart against stress-induced and ischemic damage. This means that we tried in the most economical way to form the systemic structural trace that constitutes the basis of the third stage of the process and of all the prophylactic and therapeutic effects described henceforth. As we shall see, in adaptation to hypoxia the systemic structural trace has quite ramified architectonics, encompassing many neural centers, endocrine glands, and working organs. Such a broad structural basis of adaptation to hypoxia should not be surprising, since in the whole organism hypoxia may arise not only from reduced oxygen content in the inhaled air and is not an exclusive

domain of pathology. On the contrary, in the healthy organism hypoxemia or relative tissue hypoxia inevitably and most commonly occurs in significant loads on the whole organism or on particular organs and systems. Accordingly, the organism has evolved an efficient apparatus for antihypoxic defense, and to hypoxia caused by load or reduced oxygen content in the air it responds first with immediate but unstable and then with stable long-term adaptation.

Many years of experimental studies have now led to the concept that such stable adaptation is based on at least five complexes forming an integrated systemic structural trace.

The *first complex* develops in the oxygen uptake and transport system, which responds to the lack of oxygen in the milieu with regulatory hyperfunction. In the very first days of exposure to hypoxia the formation of the systemic structural trace manifests itself as enhanced RNA and protein synthesis in the lungs,¹ heart,² bone marrow which generates erythrocytes,^{3,4} coronary vessels,⁵ and in the sympathetic neurons innervating the heart.⁶ This results in increased respiratory surface and number of lung alveoli, moderate hypertrophy and increased functional capacity of the heart, increased capacity of the coronary bed, polycythemia and increased oxygen capacity, and hypertrophy of the neurons of the respiratory centers and respiratory muscles.

Simultaneously, the power of the energy-providing system is augmented in the cells of the heart and other organs, as evidenced by the greater number of mitochondria and elevated glycolytic enzyme activity.⁵ Concurrently, changes develop in the cardiac neural regulation: hypertrophy of the sympathetic neurons of stellate ganglia and elevated adrenal content of norepinephrine with a balanced rise in cardiac phosphodiesterase.⁷ These changes, by increasing the power and efficiency of respiration and circulation and enforcing the possibility of their adrenergic mobilization, not only definitely enhance the resistance of the organism to hypoxia, but also produce the well-known cross-protective effect against physical load and potentiate training to this factor. Furthermore, adaptation to hypoxia protects the heart in myocardial infarction. It produces (1) an anti-ischemic effect, i.e., owing to collaterals the ischemic area is attenuated,⁸ and (2) a direct cytoprotective effect, i.e., a larger portion of the primarily ischemic tissue then recovers and does not undergo necrosis (see below). As a result, 1 d after infarction the infarct size proves smaller and heart contractility is better retained than in the control.

The *second complex* comprises quite a number of shifts at the higher level of neuroendocrine regulation, and involves activation of RNA and protein synthesis in the brain. In the large hemispheres this process is most pronounced in the cortex, where the RNA concentration rises by 50% and protein synthesis is doubled; in the lower brain divisions, less sensitive to the lack of oxygen, the activation is much smaller, but proves pronounced again in the vegetative centers of the medulla oblongata.⁹ In the process, the diameter of cell nuclei increases in the cortical pyramid neurons and decreases in the glial cells, i.e., there is a shift in the neuroglial relations probably associated with the donor functions of the glia.¹⁰

Simultaneously, serotonin and dopamine accumulate in the brain, while the norepinephrine content decreases somewhat; and in the adrenal glands the amount of opioid peptides, first of all beta-endorphin, increases many times over,¹¹ whereas the blood levels of serotonin and histamine decline.¹² As expected, this broad complex of stable changes in the neuroendocrine regulation has consequences that go far beyond just enhancing the tolerance to hypoxia. They show as quicker formation of conditioned reflexes and especially their better preservation,¹³ radical changes in the animal behavior in conflict situations (like rats acquiring the ability to carry out a vital drinking reflex at such electric-pain stimulation as has previously kept them away from the drinking bowl),¹⁴ or increased resistance to the epileptogenic action of a strong audio stimulus.¹⁵

Besides, the regulatory changes brought on in adaptation restrict the stress reaction and protect the heart, stomach, and liver from stress damage.^{16,17} Such protection takes place in

TABLE 1
Effect of Adaptation to Intermittent Hypoxia on the
Hepatic Cytochrome P₄₅₀ System

Indices	Control	Adapted
N-Demethylase activity (nmol/min·mg)	4.23 ± 0.19	5.57 ± 0.13*
Cytochrome b ₅ (nmol/mg)	0.50 ± 0.02	0.81 ± 0.03*
Cytochrome P ₄₅₀ (nmol/mg)	0.63 ± 0.03	1.10 ± 0.04*

* p <0.01.

a rather generalized form and is revealed, in particular, as complete prevention of marked attenuation of stress enzymemia (i.e., release of cytosolic and lysosomal enzymes into the blood commonly observed upon severe stress).¹⁸

Accordingly, with adaptation to intermittent hypoxia it proves possible to prevent not only the stress-induced drop in cardiac contractility and fibrillation threshold,¹⁹ but also ischemic arrhythmias whose neurogenic origin has been proved in numerous modern studies.^{20,21}

The *third complex* reveals itself as stable enough shifts in the regulation of water-salt metabolism and the myogenic tone of resistive vessels. Adaptation is attended with partial atrophy of the hypothalamic supraoptic nucleus and of the adrenal glomerular zone, i.e., structures that through aldosterone and the antidiuretic hormone ensure retention in the organism of a certain reserve of water and sodium chloride.^{1,22} This is accompanied by a decline in the myogenic component of the vascular tone and decreased rigidity of arteria and arterioles, which should attenuate the extent of pressor reflexes. Clearly, such shifts must cause the organism to lose the excess of water and salt and, other things being equal, must diminish the probability of hypertension. Indeed, in spontaneously hypertensive rats adaptation to hypoxia largely impedes the development of hypertension, which is rather close to the hypertensive disease of man.²³ A similar effect of adaptation is observed with deoxycorticosterone-salt hypertension.²⁴

The *fourth complex* of changes is found in the immune system, where B lymphocytes become predominant over T lymphocytes in the lymphoid organs such as spleen. An important consequence of this is partial depression of the T-mediated immune reaction and a concomitant enhancement of the humoral immune response as judged by the number of antibody-producing cells and the antibody levels in the blood.^{25,26} These adaptive changes in the immune system are the cause of certain modulatory protective effects of adaptation, namely (1) suppression of such an allergic phenomenon as adjuvant arthritis,^{27,28} (2) attenuation of the stress-induced enhancement of delayed-type hypersensitivity,²⁸ and (3) prevention of the stress depression of normal killers which are an important element of antitumor immunity.²⁹

Finally, the *fifth complex* consists in that adaptation to intermittent hypoxia substantially increases the hepatic content of cytochrome P₄₅₀-linked enzymes, acting paradoxically like chemical inducers. Table 1 shows the data of Nikonorov et al. on this issue.

This novel fact implies that the detoxicating capacity of the liver must increase in adaptation to hypoxia, and agrees nicely with the earlier reports that such adaptation appreciably enhances the tolerance to hallucinogens and epileptogens.⁵ It also explains the important data that such adaptation prevents the impairment of reflex activity and the enzymemia of injury in animals acutely or chronically exposed to hydrogen sulfide-containing gas condensate.³⁰ Further, it is essential that, besides increasing the overall cytochrome P₄₅₀ content, adaptation to intermittent hypoxia enhances the hepatic activity of cholesterol 7- α -hydroxylase, the enzyme responsible for cholesterol oxidation to bile acids. This factor,

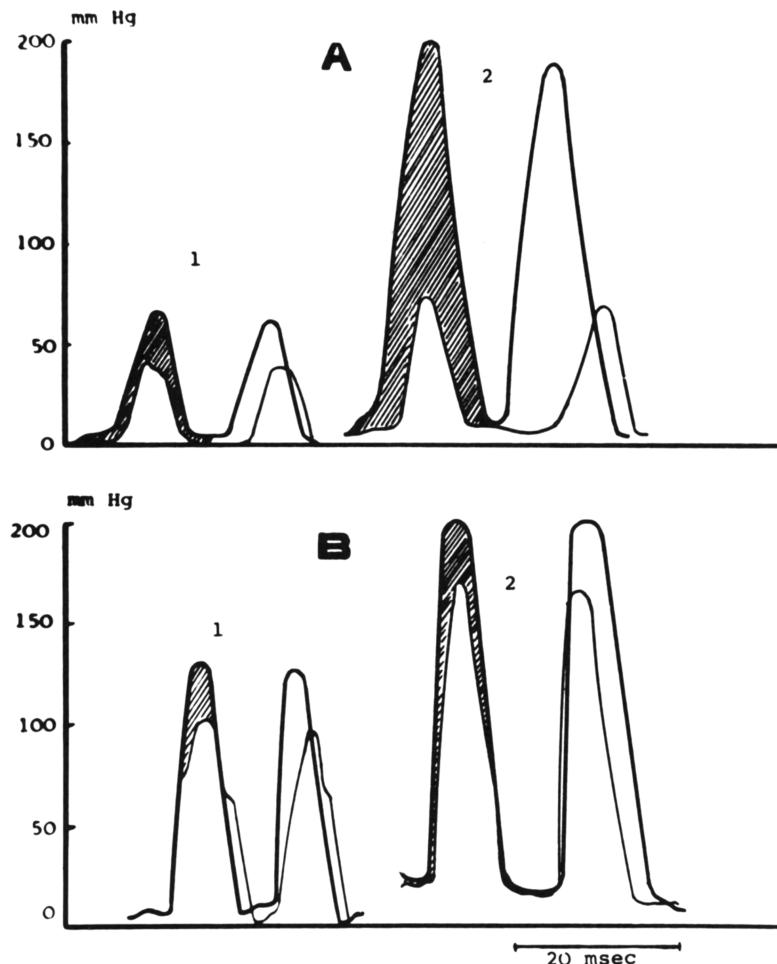


FIGURE 1. Curves of left ventricular pressure in (A) nonadapted rats and (B) rats adapted to altitude hypoxia. (1) Physiological rest, (2) aortic clamping. The shaded zone reflects the defect of contractility caused by ischemic necrosis.

together with the higher stress resistance of the organism, would naturally prevent the stress damage to the liver and thereby the stress-induced atherogenic dyslipidemia,¹⁷ which can play a weighty role in development of coronary atherosclerosis.

This presentation does not involve a detailed consideration of all possible protective effects of adaptation to hypoxia. In accordance with the main line of the book, we shall first consider two examples of the use of adaptation to intermittent hypoxia in an altitude chamber, to protect the heart from stress-induced, ischemic, and reperfusion damage, and to prevent hepatic damage and atherogenic dyslipidemia.

II. ADAPTATION TO HYPOXIA PREVENTS MYOCARDIAL STRESS AND ISCHEMIC DAMAGE AND CARDIAC ARRHYTHMIAS

It is almost 20 years since it has been quantitatively established that prior adaptation to hypoxia in an altitude chamber appreciably protects the heart against direct ischemic injury caused by coronary artery ligation.

Figure 1 shows the results of our experiments in which the cardiac contractile function

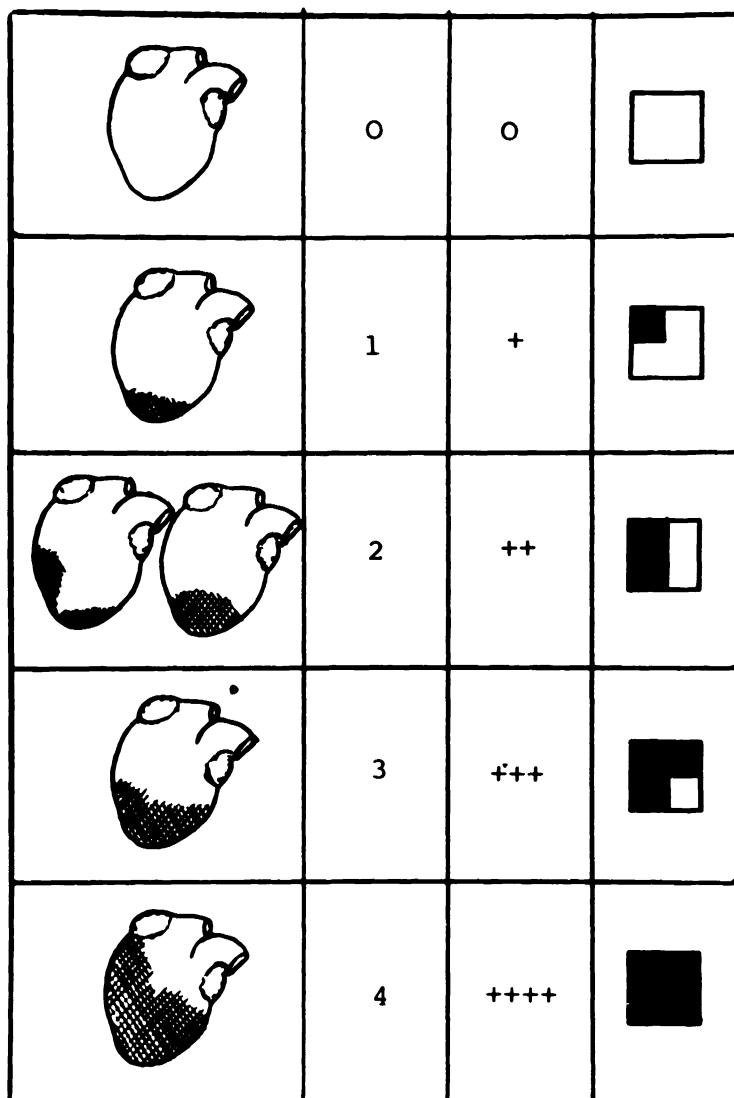


FIGURE 2. Evaluation of the extent of sympathomimetic myocardial necrosis.
 (Adapted from Poupa, O., Krofta, K., and Prochazka, J., *Fed. Proc.*, *Fed. Am. Soc. Exp. Biol.*, 25, 1243, 1966. With permission.)

and the myocardial infarct size were determined 2 d after coronary artery ligation in non-adapted rats and in rats adapted to intermittent hypoxia for 1.5 months.⁸ In these experiments the necrotic area (which at that time could be only roughly estimated) in adapted animals was reduced by 27%, whereas the depression of the maximal contraction force was three times less. Here we can recall the still earlier data of Poupa et al.;³¹ in this work the size of isoproterenol-induced myocardial necroses was expressed in arbitrary points as shown in Figure 2, and was found to be threefold smaller in hypoxia-adapted animals.

These facts per se made one optimistic in respect of using adaptation to moderate intermittent hypoxia to protect the heart from stress-induced and adrenergic damage. In all our experiments described above and henceforth the adaptation was carried out as follows: during the first 4 to 5 d the animals were "raised" first to 500, then to 1000, 2000, 3000, and 5000 m. At this "plateau" the animals had 20 to 25 hypoxic exposures of 5 to 6 h each, 6 times per week.

TABLE 2
Comparison of the Protective Effects of Adaptation to Intermittent Hypoxia and to Repeated Stress, and of the Adrenoblocker in Experimental Myocardial Infarction

Series	Relative myocardial volume (%)			
	Ischemic to total	Necrotic to ischemic	Recovered to ischemic	Necrotic to total
Control (n = 14)	36.8 ± 3.6	73.5 ± 3.8	26.5 ± 1.7	27.0 ± 1.4
Adapted to stress (n = 11)	34.3 ± 4.1	44.7 ± 4.1*	55.3 ± 2.0*	15.3 ± 1.4*
Adapted to hypoxia (n = 11)	20.9 ± 3.3*	56.0 ± 9.7*	42.2 ± 1.2*	11.7 ± 1.2*
Propranolol (n = 14)	31.9 ± 3.7	52.9 ± 4.0*	47.0 ± 1.7*	16.9 ± 1.3*

* p <0.05 compared with the control.

Together with Shneider we tried to ascertain to what extent the adaptation to hypoxia can restrict the ischemic area and enhance the myocardial resistance to ischemia, in experiments detailed in the end of the preceding chapter. The data summarized in Table 2 show that the primary ischemic area upon left coronary artery ligation is markedly reduced in animals adapted to hypoxia; this direct anti-ischemic effect was not observed with adaptation to stress or administration of propranolol.³² Further, adaptation to hypoxia also decreased the relative necrotic to ischemic area, i.e., increased the myocardial cell resistance to ischemia (although on the relative basis this cytoprotective effect was understandably smaller than with adaptation to stress or with propranolol). As assessed by the final index, namely the relative necrotic volume, adaptation to hypoxia proved to be the most effective, reducing the necrosis by a factor of 2.5. Thus, owing to combined anti-ischemic and cytoprotective action, adaptation to hypoxia appears to be one of the most if not the most efficacious means of restricting the myocardial infarct size in acute irreversible coronary artery occlusion.

It is obvious, however, that the fate of a conscious organism in such an infarction (regardless of the cause of coronary occlusion) depends, besides the infarct size, on many other factors, and first of all on the state of cardiac neural regulation, which may predetermine or, conversely, prevent arrhythmias, fibrillation, and cardiac arrest (see Chapters 3 and 4). Bearing this in mind, we shall now consider our data on the effect of adaptation to hypoxia on cardiac electric stability and arrhythmias in stress, acute ischemia, reperfusion, infarction, and postinfarction cardiosclerosis. All techniques used in these studies have been described earlier.

In the control animals, the ventricular fibrillation threshold (VFT) varied within 6.8 to 8.1 mA; vagal stimulation with quadruple-threshold currents lead to bradycardia (the heart rate dropped from 330 to 120 beats per minute, i.e., by more than 60%) attended with only occasional extrasystoles. After an immobilization stress, the VFT dropped more than twofold, to 3.4 to 3.7 mA; bradycardia was aggravated without any appreciable increase in the number of extrasystoles; there was no impairment of the contractile function. In animals preadapted to hypoxia the VFT was the same as in controls, whereas the vagal bradycardia was less than 40% and was not attended with extrasystoles; their VFT and contractility were in no way affected by the immobilization stress. Thus, the widely known phenomenon of a poststress decline in the cardiac fibrillation threshold³³ was completely prevented by prior adaptation to hypoxia.

The antiarrhythmic action of adaptation to hypoxia in acute ischemia is most pronounced in conscious animals, less pronounced under anesthesia, and insignificant in isolated hearts (Figure 3); in reperfusion, it decreases the duration of grave arrhythmias by half in all three cases. These data prompt the idea that its protective action involves mainly central mech-

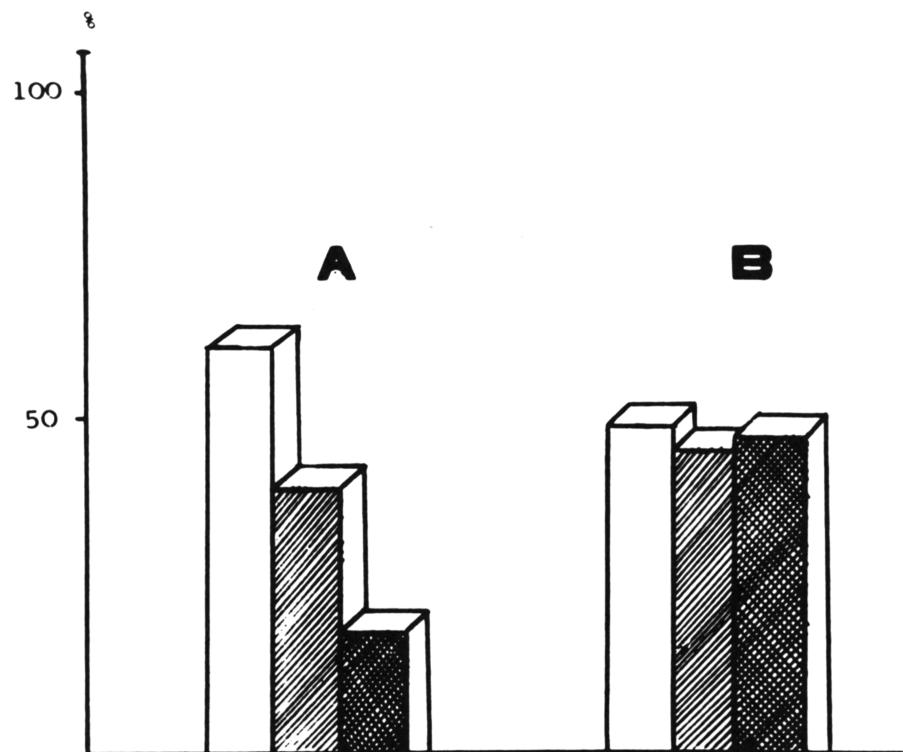


FIGURE 3. Antiarrhythmic effect of adaptation to hypoxia on (A) ischemic and (B) reperfusion arrhythmias in conscious animals (empty blocks), anesthetized animals (hatched blocks), and isolated hearts (cross-hatched blocks); the block height reflects the decrease in duration of grave arrhythmias (ventricular tachycardia plus ventricular fibrillation).

TABLE 3
Effect of Myocardial Infarction and Adaptation to Hypoxia on the Electric Stability and Contractile Function of the Heart

Indices	Control	Infarction adapted (n = 10 each series)	Adapted + infarction
Ventricular fibrillation threshold	5.3 ± 0.2	2.0 ± 0.3*	5.2 ± 0.3
Number of extrasystoles in vagal bradycardia	0	124	0
Developed pressures			
At rest	110 ± 9	52 ± 10*	117 ± 8
5 s of aortic clamping	208 ± 18	121 ± 17*	215 ± 35
30 s of aortic clamping	220 ± 21	116 ± 17*	240 ± 21

* Difference with "control" $p < 0.05$.

† Difference with "infarction" $p < 0.05$.

anisms in ischemia and mainly local cardiac mechanisms in reperfusion (see also Chapter 6, Section II).

The data presented in Table 3 demonstrate the influence of prior adaptation to hypoxia on the electric stability and the contractile function of the heart in infarction. As can be seen, adaptation virtually completely prevents the drop in the VFT. Further, the pronounced

TABLE 4
Effect of Postinfarction Cardiosclerosis and Adaptation to Hypoxia on the Electric Stability and Contractile Function of the Heart

Indices	Cardiosclerotic		Adapted	Cardiosclerotic adapted
	Control	(n = 10 each series)		
Ventricular fibrillation threshold	8.1 ± 0.3	3.7 ± 0.7 ^a	7.9 ± 0.4	6.4 ± 0.4 ^b
Number of extrasystoles in vagal bradycardia	0	78	0	0
Developed pressure				
At rest	95 ± 4	65 ± 9 ^a	113 ± 4 ^a	88 ± 8 ^b
5 s of aortic clamping	150 ± 7	137 ± 18	181 ± 9 ^a	163 ± 12
30 s of aortic clamping	159 ± 7	62 ± 10 ^a	181 ± 10 ^a	149 ± 14 ^b

^a Difference with "control" $p < 0.05$.

^b Difference with "cardiosclerotic" $p < 0.05$.

ectopic activity during vagal bradycardia observed in infarction is largely (2.3-fold) attenuated by adaptation. Thus, *prior adaptation to intermittent hypoxia substantially reduces the probability of extrasystoles and cardiac fibrillation in experimental myocardial infarction*.

When the contractile function of the heart was studied at physiological rest and under maximal load, the animals with myocardial infarction displayed the well-known phenomenon — a 40 to 50% depression of the developed pressure. The adaptation, itself only slightly increasing this index, reliably diminished its decline in infarction.

Thus, adaptation to intermittent hypoxia definitely restricts the impairment of cardiac electric stability and contractility in acute myocardial infarction.

In postinfarction cardiosclerosis, 2 months after infarction, there is no stress or ischemia; therefore, a study of the effect of adaptation in this model makes it possible to distinguish its antiarrhythmic action proper from the antistress and anti-ischemic ones. Table 4 shows that in postinfarction cardiosclerosis the VFT is more than twice lower and there are numerous extrasystoles in vagal bradycardia. Adaptation carried out in preexisting cardiosclerosis restores the VFT to the control level and eliminates the vagally evoked extrasystoles, thus reducing the probability of cardiac fibrillation. Further, Table 4 shows that in cardiosclerotic animals adapted to hypoxia the developed pressure after 30 s of aortic clamping remains as high as at the fifth second.

Thus, adaptation to hypoxia abolishes the disturbances of cardiac electric stability and contractility typical of postinfarction cardiosclerosis.

To elucidate the structural basis of this therapeutic effect, we undertook a morphometric study of the relative scar volume, relation of formed elements in the cicatrix, and vascularization of the adjacent myocardial tissue.

Vascularization and formed elements were quantitated using stereometric grids.³⁴ The scar size was determined with serial histotopographic sections of the heart cut at every 2 mm starting from the apex. The relative scar volume was calculated in accordance with the principle of Dellesse that the cross-section areas of structures relate as their volumes.³⁵

These results are shown in Table 5: adaptation to hypoxia reduces the relative scar volume by about one third, the cicatrix itself becomes denser with a greater proportion of collagen fibers and a lesser proportion of edematous stroma and all formed elements. Simultaneously, the amount of vessels in the myocardial tissue adjacent to the cicatrix increases by one third.³⁶

By and large, adaptation to hypoxia restricts the infarct size in acute coronary artery occlusion, and reduces the size of the preexisting postinfarction cicatrix.

It is clear, however, that these cardiac effects of adaptation would not themselves be able to ensure its powerful antiarrhythmic effect definitely shown in our experiments.

TABLE 5
Relations Among Histological Structures
in the Postinfarct Cicatrix and Adjacent
Myocardium (%)

Indices	Control	Adapted
Relative volume of scar tissue	25.2	18.7
Vascularization of border zone	16.5	21.7
Edematous stroma	1.5	0.5
Collagen fibers	56.0	65.2
Fibroblasts	5.1	4.2
Fibrocytes	5.2	8.0
Polymorphonuclear leukocytes	0.5	None
Lymphocytes	11.2	8.1
Plasmacytes	8.6	5.2
Eosinophils	0.7	0.3
Macrophages	10.2	7.5

TABLE 6
Effect of Prior Adaptation to Hypoxia on Brain and
Adrenal Beta-Endorphin Contents in Stress

Series (n = 10 each)	Beta-endorphin (fmol/mg tissue)			
	Cortex	Striatum	Cerebellum	Adrenals
Control	2.4 ± 0.7	5.7 ± 1.8	2.1 ± 0.4	1.2 ± 0.3
EPS	0.6 ± 0.1	1.3 ± 0.3	1.4 ± 0.7	0.8 ± 0.3
Adapted	2.8 ± 0.7	7.0 ± 2.2	2.9 ± 0.5	5.1 ± 0.2
Adapted + EPS	2.1 ± 0.8	5.5 ± 2.1	3.0 ± 1.1	0.6 ± 0.1

The fact that adaptation to hypoxia is effective against both ischemic and reperfusion arrhythmias (see above) prompts one to think that its antiarrhythmic action should involve both central and local stress-limiting systems.

To elucidate the role of the central regulatory mechanisms in the antiarrhythmic effect of adaptation, we determined the brain and adrenal contents of the most active opioid peptide, beta-endorphin, in adapted and nonadapted animals in normal conditions and after a severe EPS. Adaptation to hypoxia was carried out in an altitude chamber at 5000 m for 8 weeks, 5 times a week, 6 h a day.

Table 6 shows that EPS decreases the endorphin content in the cortex, striatum, and cerebellum by a factor of 1.5 to 4; adaptation, which itself only slightly increases the endorphin content in the brain structures, at the same time virtually prevents its changes in stress. To understand this remarkable ability of the brain of adapted animals to maintain the normal endorphin concentration despite stress, attention should be paid to the last column in Table 6. There one can see that in the course of adaptation the adrenal concentration of beta-endorphin increases over fourfold. Mobilization of this large surplus in stress proves to be even more complete than in nonadapted animals. As a result, *the stress-induced discharge of beta-endorphin from the adrenal glands of adapted animals exceeds the control one by more than an order of magnitude. Since endorphins check the excitation of the brain adrenergic structures important in arrhythmias and cardiac fibrillation, and also act as analgetics, it is easily conceivable that increased power of the opioid system in adaptation to hypoxia is a factor preventing cardiac fibrillation in acute ischemia.*

It is noteworthy that there is a quantitative correlation between these data and our previous work³⁷ on the increased adrenal content and stress-induced discharge of catecholamines in

TABLE 7
Effect of Adaptation to Hypoxia on the
Neurotransmitter Contents in the Brain Cortex
(μ g/g tissue)

Compound	Control (n = 5)	Adapted (n = 10)	p
Norepinephrine	186 \pm 5	150 \pm 9	<0.05
Dopamine	132 \pm 5	167 \pm 5	<0.05
Serotonin	108 \pm 4	238 \pm 8	<0.01
5-Hydroxyindolyl acetic acid	173 \pm 53	270 \pm 9	<0.01

adaptation to hypoxia. Thus, adaptation increases the stress discharge of beta-endorphin from 0.44 to 4.49 fmol/mg and that of norepinephrine from 40 to 400 ng/mg of the adrenal gland, i.e., by the same factor of 10. This corresponds to the known fact that catecholamines and opioid peptides checking their effect are synthesized in the same granules, and to our concept of the intimate coupling between the stress-effecting and the stress-limiting systems.

In Chapter 4 it has been shown in detail that the opioid system exerts a many-sided influence on the brain monoaminergic system and, in particular, can activate the serotonergic system involved in attenuation of arrhythmias. Therefore, we suggested that changes in the monoamine metabolism in the brain may also contribute to the antiarrhythmic effect of adaptation to hypoxia.

To check this suggestion, the frontocortical contents of norepinephrine, dopamine, serotonin, and 5-hydroxyindolylacetic acid were determined spectrofluorimetrically after column resolution according to Earley and Leonard.³⁸ As shown in Table 7, norepinephrine decreased by 35% while dopamine increased by 26%, serotonin by 120%, and its breakdown product by 60%.

A possible explanation of these results stems from the concept of coordination of the opioidergic and the serotonergic systems and their combined action to restrict the arrhythmogenic influence of adrenergic centers on the heart, as detailed in Chapter 4. This tentative explanation pertains mainly to stress-induced and ischemic arrhythmias of central genesis, but is much less applicable to the adaptational protection against reperfusion arrhythmias and impairment of the electric stability in postinfarction cardiosclerosis; in these cases a prominent part is most likely played by the local stress-limiting systems such as the adenosinergic, prostaglandin, and antioxidant ones.

The latter is favored by the recent observation of enhanced resistance to the arrhythmogenic action of a lipid peroxidation (LPO) inducer, H₂O₂, in the isolated auricles from animals adapted to hypoxia (work done by Saltykova and Ustinova). Figure 4 compares the response of 20 control and 20 "adapted" auricles to H₂O₂. As can be seen, in the control occasional auricles stopped contracting already in 1 to 2 min (adapted ones only in 3 to 4 min), and by the 20th min only 2 control, but 12 adapted auricles retained their spontaneous activity. Thus, *adaptation to hypoxia enhances the resistance of cardiac automatism to the arrhythmogenic action of an LPO inducer*. It should be remembered that catecholamines, which injure the heart in stress and acute ischemia, are also LPO inducers and at the same time have a pronounced arrhythmogenic effect.

In the aggregate, the unambiguous evidence available for the powerful cardioprotective (and in particular, antiarrhythmic) effect of adaptation to intermittent hypoxia appears a sound enough basis for using this approach to treat certain types of arrhythmia in man.

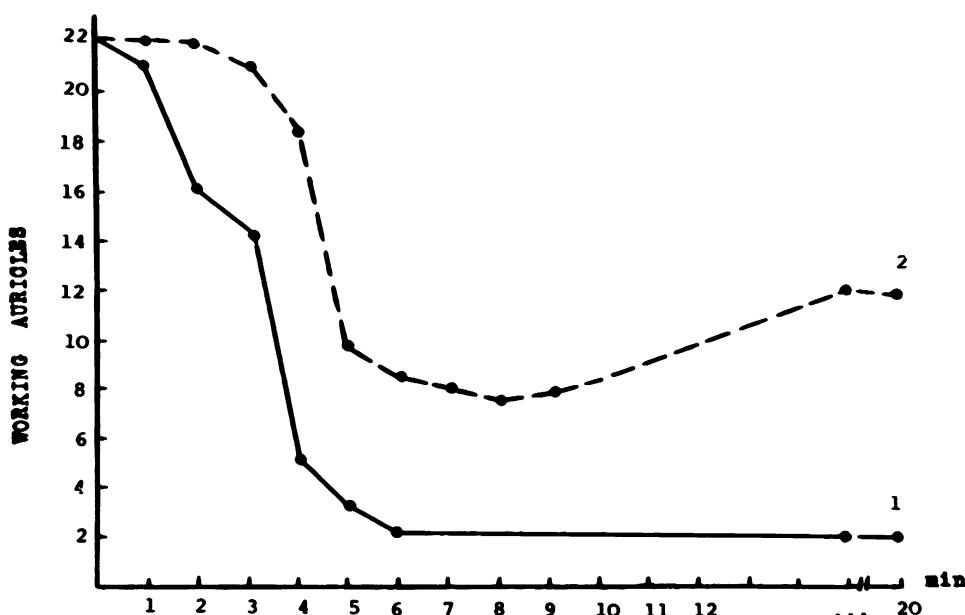


FIGURE 4. Effect of adaptation to hypoxia on the rat atrial resistance to the arrhythmogenic action of hydrogen peroxide. (1) Control, (2) adapted.

III. ADAPTATION TO INTERMITTENT HYPOXIA PREVENTS HEPATIC STRESS DAMAGE AND ATHEROGENIC DYSLIPIDEMIA

The concept of the weighty role of excessive consumption of food cholesterol in the pathogenesis of atherosclerosis^{39,40} is now generally recognized. Further studies have shown that central to the development of atherosclerosis (caused by alimentary or any other factors) are the disturbances in lipid turnover in the liver, which is the key organ of cholesterol metabolism. A genetic insufficiency of hepatic receptors that bind the plasma cholesterol in high-density lipoproteins (HDL)⁴¹ and/or abnormalities in the microsomal system of cholesterol oxidation and elimination,^{42,43} and probably some other shifts in the hepatic metabolism ultimately lead to atherogenic dyslipoproteinemia, which is highly conducive to cholesterol deposition in the vascular wall and development of atherosclerosis.

The important part played by hepatic stress damage, and specifically impaired cholesterol oxidation to bile acids, in atherogenic dyslipidemia and atherosclerosis of coronary vessels has already been demonstrated in this book (see Chapters 1 and 2). Here we shall consider our recent results obtained together with Tverdokhlib, which show that hepatic stress damage and atherogenic dyslipidemia can be prevented with prior adaptation to intermittent hypoxia.

As can be seen in Figure 5, adaptation to hypoxia substantially increases the activity of the antioxidant enzyme superoxide dismutase (SOD) and prevents the activation of LPO (as judged by the malonic dialdehyde [MDA] content) in prolonged emotional pain stress (EPS).

Further, it has been found that the adaptation enhances mitochondrial DNA replication in the liver and, what is most important, appreciably attenuates both the decline in nuclear DNA replication and the outburst of DNA repair upon EPS (Table 8), which indicates a lesser degree of DNA damage in stress after adaptation to hypoxia.

The hepatoprotective effect of adaptation to hypoxia is also confirmed by a much smaller extent of stress-induced leakage of the liver-specific enzyme fructose-1-phosphate aldolase into the blood (Figure 5B). Furthermore, such protection against stress also covers the

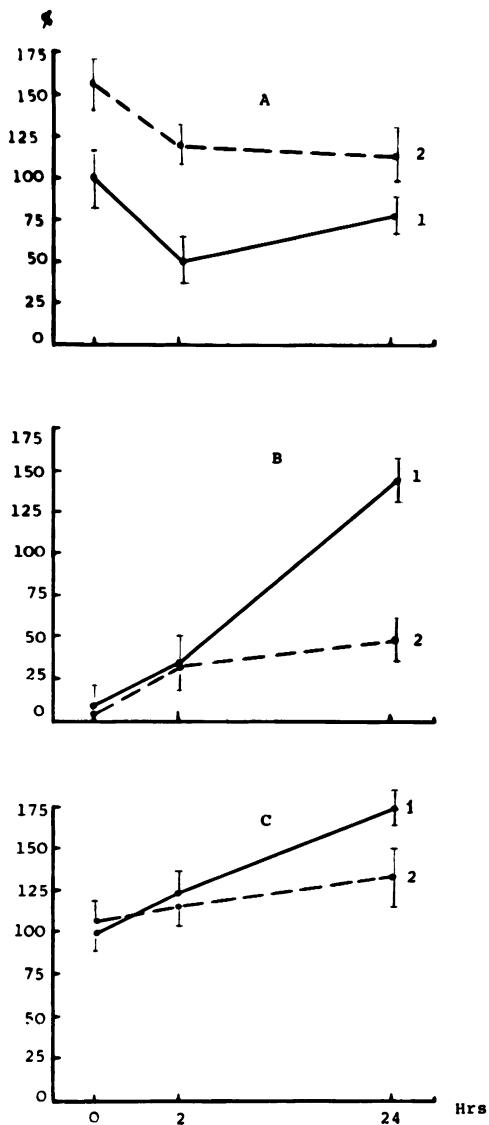


FIGURE 5. Effect of adaptation to hypoxia on (A) superoxide dismutase, (B) fructose-1-phosphate aldolase, and (C) malonic dialdehyde in EPS. (1) Control, (2) adapted. The abscissa is time after EPS; the ordinates are in percent.

microsomal enzyme system. Adaptation to intermittent hypoxia elevates the hepatic cholesterol 7- α -hydroxylase activity and prevents its decline in prolonged stress (Figure 6). Cholesterol hydroxylase plays a key role in cholesterol elimination from the organism; hence, preservation of this enzymic activity in adapted animals allows one to expect attenuation of stress-induced atherogenic dyslipidemia.

The effect of prior adaptation to intermittent hypoxia on the extent of stress-induced atherogenic alterations in plasma lipoproteins is shown in Table 9. As can be seen, such adaptation indeed has a pronounced antiatherogenic effect. First, cholesterol is redistributed among the lipoprotein fractions (increased in HDLP and decreased in low-density lipoproteins [LDLP] and VLDLP) so that the atherogenicity index in adapted animals is markedly lower

TABLE 8
Effect of Adaptation to Hypoxia on Stress-Induced
Alterations in Hepatic DNA Replication and
Repair Assayed by ^3H -Thymidine Incorporation
(cpm/mg DNA)

Series	Nuclear DNA repair	DNA replication	
		Nuclear	Mitochondrial
Control	3,700	27,000	38,000
EPS	6,500	16,000	22,100
Adapted	3,950	25,200	52,000
Adapted + EPS	5,000	20,300	40,000

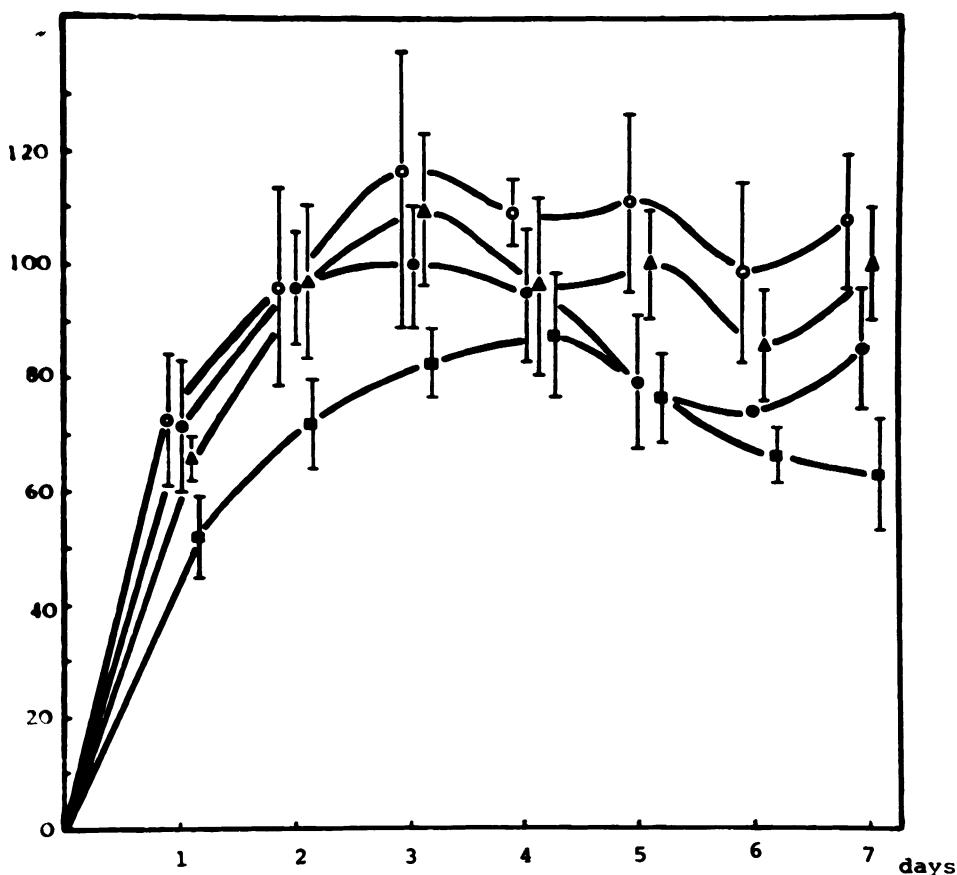


FIGURE 6. Effect of adaptation to hypoxia on ^3H -cholesterol oxidation after EPS in rats. (●) Control, (■) EPS, (○) adapted, (▲) EPS in adapted animals.

than in the control. Second, the dyslipoproteinemia caused by severe EPS is much abated by such adaptation, and the atherogenicity index still does not exceed the normal value.

Thus, rather mild adaptation to intermittent dosed hypoxia can be used as an active means of preventing stress-induced atherogenic disturbances.

Taken together with the above data on induction of cholesterol hydroxylase (Figure 6) and P_{450} -linked enzymes (Table 1), all this in essence means that *such adaptation can prevent*

TABLE 9
Indices of Lipid Metabolism in the Serum of Nonadapted and Hypoxia-Adapted Rats

Indices	Control	EPS	Adapted	Adapted + EPS
(n = 20 each series)				
Total cholesterol	69.5 ± 3.6	58.6 ± 4.5	73.6 ± 2.9	63.8 ± 1.7
Triglycerides	52.8 ± 4.2	40.8 ± 3.2*	31.2 ± 3.6*	22.9 ± 2.8*
HDLP cholesterol	52.6 ± 3.9	22.7 ± 1.7*	63.6 ± 2.8*	45.9 ± 2.8*
VLDLP cholesterol	10.6 ± 0.5	8.1 ± 0.7*	6.2 ± 0.7*	4.6 ± 0.6*
LDLP cholesterol	8.5 ± 1.4	27.3 ± 4.0*	4.8 ± 1.6*	10.1 ± 1.8*
Atherogenicity index	0.4	1.6	0.15	0.35

* p <0.05.

or abolish atherogenic dyslipidemias of quite different etiology. Obviously, such a means of reducing this serious risk factor of ischemic disease compares favorably with the radical solutions used today like repeated hemosorption, or liver transplantation to children with hereditary atherogenic dyslipidemia and rapidly advancing coronary atherosclerosis.⁴⁴ Thus, there are full grounds for undertaking clinical studies of the practicability and effectiveness of adaptation to intermittent hypoxia.

IV. DIFFERENCES OF ADAPTATION TO INTERMITTENT HYPOXIA FROM ADAPTATION TO CONTINUOUS HYPOXIA, AND THE TACTICS OF ITS APPLICATION

It must be accentuated that adaptation to intermittent hypoxia in the altitude chamber has a number of essential differences from adaptation to continuous hypoxia in the mountains.

1. According to our tactics, the "rise" (i.e., the reduction of oxygen tension in the air) takes place gradually: to 500 m the first day, 1000 m the second, and so on to 3500 m, actually following the principle of stepwise adaptation, whereas even in "gentle" adaptation to mid-mountain conditions people, by established routine, are directly brought to an altitude of 2000 m which is considered easily tolerable. This difference is not just a technical one, since the initial emergency stage of adaptation to any factor is inevitably attended with a stress reaction and its catabolic effect (i.e., involves substantial structural costs), regulatory hypertension, and the general malaise syndrome. Adaptation to intermittent hypoxia offers a practicable possibility of minimizing the stage or avoiding it altogether.
2. In the intermittent mode, the course usually consisting of 30 to 40 seances actually represents not only adaptation to hypoxia, but also 30 to 40 instances of slow but inevitable reoxygenation, each of which should naturally cause some activation of free-radical lipid oxidation and, accordingly, of the enzymic and nonenzymic antioxidant mechanisms. In full conformity with this, adaptation to intermittent hypoxia has been shown to enhance the activity of the antioxidant enzymes SOD and catalase in the brain and in the liver.⁴⁵ Together with Didenko and Arkhipenko we have recently compared the antioxidant enzyme activities in adaptation to intermittent and to continuous hypoxia. In adaptation to intermittent hypoxia in the altitude chamber, the SOD and catalase activities increased by 10 to 12% in the heart, by 38 and 29%, respectively, in the brain, and by 47 and 28% in the liver. In contrast, adaptation to continuous hypoxia (carried out at the Terskol Canyon in the Caucasus, altitude 2100

TABLE 10
Effects of Adaptation to Continuous and Intermittent Hypoxia on the Duration of Grave Arrhythmias and Mortality in Acute Ischemia and Reperfusion

Series, number, and weight of animals	Ischemia		Reperfusion	
	VT + VF*	Deaths	VT + VF*	Deaths
1. Control I (n = 10, 200–220 g)	21 ± 5	0	42 ± 10	0
2. Acute hypoxia (n = 12, 200–220 g)	130 ± 20	1	142 ± 36	2
3. Continuous, 7d (n = 10, 240–260 g)	117 ± 19	0	259 ± 13	4
4. Continuous, 16 d (n = 11, 280–300 g)	76 ± 36	0	228 ± 25	4
5. Continuous, 30 d (n = 11, 360–380 g)	35 ± 7	0	286 ± 25	5
6. Control II (n = 11, 360–380 g)	96 ± 6	0	184 ± 19	3
7. Intermittent, 30 d (n = 11, 360–380 g)	58 ± 5	0	69 ± 7	0
<i>P</i> ₁₋₂	<0.001		<0.001	
<i>P</i> ₁₋₃	<0.001		<0.001	
<i>P</i> ₁₋₄	<0.05		<0.001	
<i>P</i> ₅₋₆	<0.001		<0.05	
<i>P</i> ₆₋₇	<0.05		<0.001	

* VT, ventricular tachycardia; VF, ventricular fibrillation.

m) caused decreases in these activities: 35% for SOD and 15% for catalase in the heart, 33 and 23%, respectively, in the brain, and 31 and 19% in the liver. Hence, with respect to antioxidant activity, adaptation to intermittent and to continuous hypoxia produce opposite effects. Table 10 shows that such differences prove dramatic for animals tested for cardiac resistance to reperfusion arrhythmias (experiments of Vrontsova and Ustinova). In 30 d, either type of adaptation decreased the total duration of grave ischemic arrhythmias (ventricular tachycardia plus ventricular fibrillation); however, in reperfusion arrhythmias (where LPO is pathogenetically decisive) the situation was essentially different: adaptation to continuous hypoxia increased the duration of reperfusion arrhythmias and raised the mortality to almost 50%, whereas adaptation to intermittent hypoxia significantly shortened the duration of and abolished mortality from reperfusion arrhythmias.

This particular case gives grounds for a more general idea that adaptation to intermittent hypoxia can be protective against both ischemic and reperfusion damage, while adaptation to continuous hypoxia, by lowering the antioxidant potential, aggravates the reperfusion injury and hence all types of reoxygenation damage rather than protects against them. A prolonged stay in the mountains, through a number of mechanisms, appears to make the organism "unused to the plain"; after coming down, this reveals itself as decreased working ability and general fatigue, which the researchers justly call the "deadaptation syndrome". In adaptation to intermittent hypoxia, the subjects spend no more than 3 to 5 h in the altitude chamber and all the rest of their time at normal oxygen tension, whereby the deadaptation syndrome is certainly ruled out.

- Taking under any conditions only a small part of the day, adaptation to intermittent hypoxia leaves a healthy or a diseased person quite enough time for dosed adaptation to other factors, such as physical load, cold, etc. Under continuous hypoxia in the mountains, such combination of treatments to form harmonious adaptation may prove an excessive burden on the organism.
- Adaptation to intermittent hypoxia in the altitude chamber allows such strict rationing of the hypoxic exposure as is unrealistic in the mountains. Indeed, roughly defining

the dosage of hypoxic exposure as the product of duration by "intensity" (i.e., altitude), we can say that during a round-the-clock stay even at 1 km the subject gets a hypoxic exposure of 24 arbitrary units, while a 3-h seance at "3.5 km" actually used for adults in this work gives 10.5 units. Regardless of whether or not such calculations are valid, clearly, with the altitude chamber the risk of overdosage is much less than in continuous adaptation in the mountains. This circumstance is quite important, since overexposure to continuous and even intermittent hypoxia may cancel out the beneficial effect of adaptation, e.g., on the cardiac contractile function,⁴⁶ and even lead to progressive cardiosclerosis, mainly in the right ventricular myocardium.³¹ Further, prolonged hypoxia elevates the pressure in the pulmonary artery;^{47,48} therefore, minimization of this adaptive hypertension in the lesser circulation by restricting the duration of hypoxic exposure and with the use of nifedipine is an important feature of the tactics of adaptational therapy and prophylaxis.

5. Finally, unlike adaptation in the mountains, the state of adaptation to intermittent hypoxia can be easily sustained for extended periods with "maintenance visits" to the altitude chamber two to three times a week. In just this way we have succeeded, for example, in stabilizing the antihypertensive effect of adaptation in animals with hereditary hypertension.²³

As regards the general tactics, it should be borne in mind that just as with any other type of adaptation, this process and especially its initial stage requires maintenance of an optimal vitamin balance in the organism. This first of all applies to the group of precursors and cofactors for nucleic acid and protein synthesis: orotic and folic acids, vitamin B₁₂, methionine, lysine, etc. For instance, it has been shown that in adaptation of animals to hypoxia, besides a rise in blood hemoglobin, there is a release from the bone marrow of immature erythrocytes — reticulocytes. Administration of a complex of B₁₂, folic, and orotic acids during adaptation prevents this reticulocytosis as the bone marrow in this case can ensure complete maturation of all erythrocytes generated.⁴⁹ Equally necessary are sufficient amounts of vitamins A, C, and E.

Another quite essential aspect is the great individual variations in tolerance to hypoxia, which most likely have their molecular basis in the functional arrangement of the mitochondrial respiratory chain.⁵⁰⁻⁵³ This makes it necessary to determine the hypoxic tolerance in individuals before using such adaptation with therapeutic or preventive purposes; thus, respiratory and circulatory responses to oxygen-depleted gas mixtures should be recorded, the greater reaction being indicative of the lower tolerance to hypoxia.

In the aggregate, the above provides grounds for applying the experimentally proven great potential of adaptation to dosed intermittent hypoxia in the altitude chamber in prevention and treatment of, first and foremost, those diseases that are difficult or impossible to cure with contemporary pharmacological means.

We have used such adaptation to reduce the risk factors of ischemic disease, to abolish grave neurasthenic syndromes and associated arrhythmias, and to treat some other diseases. This clinicophysiological study has been the subject of a special joint publication.*

V. REGIMEN AND ORGANIZATION OF ADAPTATION TO INTERMITTENT HYPOXIA IN THE "MOUNTAIN CLIMATE HALL"

The medical altitude chamber *Ural-1* has been described in detail elsewhere, and its appearance is shown in Figure 7. It consists of the low-pressure chamber proper (the "hall")

* Meerson, F. Z., Tverdokhlib, V. P., Boev, V. M., and Frolov, B. A., *Adaptation to Intermittent Hypoxia in Therapy and Prophylaxis* (in Russian), Nauka, Moscow, 1989.

FIGURE 7. The medical multiseater altitude chamber *Ural-1*.



and remote evacuation and conditioning system providing "ascent" and "descent" rates of 2 to 10 m/s. Such altitude chambers should be installed at polyclinics, rehabilitation centers, and clinical research institutions.

Prior to, in the course of, and after adaptation the patients were examined in a specialized Hypobaric Therapy Unit, in clinical departments such as cardiological, neurological, etc., and in the functional diagnosis labs — biochemical, immunological, etc. The results of examinations were forwarded to a panel of experts. At the very first stage, the patients were screened for the following contraindications:

1. Ischemic heart disease, III-degree hypertensive disease, valvular defects, and cardiac failure
2. Impaired brain circulation, craniocerebral injury
3. Grave bronchial asthma
4. Exacerbated chronic and acute inflammatory processes in the lungs
5. Diffuse pneumosclerosis, extensive pleural adhesions, pronounced emphysema with symptoms of pulmonary or cardiac insufficiency
6. Active foci of infection; exacerbation of chronic tonsillitis, sinusitis, cholecystitis, etc.
7. Inflammatory lesions of middle ear and accessory nasal sinus
8. Diseases affecting the permeability of eustachian tubes
9. Pregnancy; hemorrhage-prone uterine fibromyoma
10. Decompensated diabetes mellitus
11. Hernia of any localization
12. Manifestations of hepatorenal insufficiency
13. All psychoneurotic diseases associated with improper behavior during treatment
14. Lack of psychoemotional "readiness" and negative attitude to the particular treatment

This very exacting list of contraindications served primarily to avoid complications, and can be made more flexible in the future.

VI. EFFECT OF ADAPTATION TO INTERMITTENT HYPOXIA ON TOLERANCE OF APPARENTLY HEALTHY UNTRAINED SUBJECTS AND ON SOME RISK FACTORS OF ISCHEMIC HEART DISEASE*

The subjects were apparently healthy untrained males aged 35 to 49, total number 22, selected in the outpatient department of a rehabilitation center. All men in this group displayed characteristic signs of the detrained state: decreased tolerance to substantial physical loads, excess weight, headaches, cardialgias, and impaired sleep. Still, they were all working and showed no IHD symptoms in the veloergometric test.

The course of adaptation to hypoxia reduced the mean body weight by 2 kg, which is in line with the observations that such adaptation hypothalamically checks the appetite. The sleep was normalized, blood pressure reduced, and complaints of headaches and heartaches disappeared in all subjects; 5 of 11 smokers dropped the habit.

Table 11 demonstrates the effect of adaptation on the basic circulation parameters at rest and under maximal veloergometric load. Adaptation increased the total amount of work performed by 27% and the maximal output by 15%, with the oxygen consumption at rest decreased by 20% and the maximal consumption remaining the same. The systolic and the diastolic pressure also decreased both at rest and at peak load. As a result, the double product

* Participants in the work: Ya. I. Kots, V. A. Grigor'evskikh, O. I. Tikhomirov, I. A. Aleshin, O. A. Altukhov, S. V. Perepelkin, R. E. Funin, V. B. Volovich, T. V. Krasnova, N. N. Setko, S. M. Shashchineider, and M. M. Pavlova.

TABLE 11
Effect of Adaptation to Intermittent Hypoxia on Circulation and Physical Endurance

Indices	Before adaptation	After adaptation
Heart rate (min^{-1})		
Rest	69.1 ± 1.4	61.2 ± 1.7^a
Load	152.6 ± 3.9	138.4 ± 6.0^a
Arterial pressure (mmHg)		
Systolic		
Rest	147.1 ± 1.4	117.6 ± 1.7^a
Load	205.9 ± 7.2	183.4 ± 5.1^a
Diastolic		
Rest	93.3 ± 2.1	79.1 ± 2.1^a
Load	101.6 ± 3.7	87.7 ± 7.6
Double product (AP _s ·HR/100) at peak load	314.9 ± 11.4	249.5 ± 13.1^a
Total work (kJ)	71.5 ± 6.5	93.0 ± 8.6^a
Maximal power (W)	135.5 ± 8.8	155.5 ± 6.7
Oxygen consumption (ml/min)		
At rest	5.4 ± 0.6	4.4 ± 0.2
Maximal	21.3 ± 1.9	22.4 ± 1.7

* $p < 0.05$.

at peak load was 21% lower; with the higher maximal output, the efficiency of cardiac function under load was enhanced almost by a factor of 1.5.

Thus, *adaptation to intermittent hypoxia, without any additional measures, improves the performance of untrained subjects and enhances the efficiency of the heart which often limits the maximal load.*

It is known that in the detrained state decreased tolerance to physical load is usually accompanied by decreased functional capacity of the external respiration. Our studies have shown that adaptation to intermittent hypoxia increases the forced vital capacity and the forced exhaled volume by 20% and the maximal pulmonary ventilation by 40%.

Thus *in adaptation to intermittent hypoxia the enhanced efficiency of circulation combines with the greater functional capacity of external respiration; this apparently must contribute to the improved performance under maximal load.*

One of the criteria for assessing the tolerance to physical load is the extent of enzymemia, i.e., leakage of enzymes into the blood caused by cell membrane damage. Such events in stress¹⁸ and physical loading⁵⁴ of untrained persons are in great measure determined by LPO activation; accordingly, such activation is a factor limiting the tolerable load, while administration of antioxidants to suppress LPO increases the tolerated intensity and duration of the load.⁵⁵ Table 12 demonstrates that adaptation to hypoxia lowers more than by half the blood serum levels of asparagine aminotransferase (AST) and alanine aminotransferase (ALT) at rest, and appreciably attenuates (AST) or virtually abolishes (ALT) their increase under maximum load; this probably results from the greater stability of cell membranes in the adapted organism. Quite in line with the idea of the cytoprotective role of the antioxidant mechanisms, Table 12 further shows that adaptation increases the serum content of the copper-containing protein ceruloplasmin, which has antioxidant activity,⁵⁶ and the SOD activity in the erythrocytes; concomitantly the amount of LPO products — MDA and dienic conjugates — is lowered to roughly the same extent. *Thus, it can be thought that enhanced activity of the natural antioxidant systems of the organism is one of the factors restricting the enzymemia and increasing the physical endurance in adaptation to hypoxia.*

Further analysis of the data obtained has revealed that adaptation to intermittent hypoxia

TABLE 12
Effect of Adaptation to Intermittent Hypoxia on the Blood
Biochemical Indices

Indices	Before adaptation	After adaptation
Aspartate aminotransferase (nmol/ml·h)		
Rest	640 ± 12	280 ± 45 ^a
Load	1040 ± 110	490 ± 40 ^a
Alanine aminotransferase (nmol/ml·h)		
Rest	610 ± 10	320 ± 50 ^a
Load	1110 ± 110	390 ± 50 ^a
Superoxide dismutase (U)	110 ± 4	122 ± 3 ^a
Ceruloplasmin (mg/l)	250 ± 15	315 ± 12 ^b
Malonic dialdehyde (nmol/ml)	6.8 ± 0.4	5.6 ± 0.3 ^c
Dienic conjugates (OD U/mg lipid)	0.87 ± 0.09	0.66 ± 0.04 ^c

^a $p < 0.01$.

^b $p < 0.001$.

^c $p < 0.05$.

TABLE 13
Effect of Adaptation to Intermittent Hypoxia on the
Extent of Atherogenic Dyslipoproteinemia in Men (age 34
to 49) with Initial Cholesterol over 230 mg/dl

Indices	Before adaptation	After adaptation	<i>p</i>
Total cholesterol (mg/dl)	268 ± 4 (n = 15)	239 ± 7 (n = 15)	<0.05
HDLP cholesterol (mg/dl)	46 ± 2 (n = 15)	50 ± 3 (n = 14)	
LDLP cholesterol (mg/dl)	184 ± 10 (n = 15)	163 ± 10 (n = 14)	<0.05
Triglycerides (mg/dl)	188 ± 24 (n = 15)	129 ± 17 (n = 15)	<0.05
Atherogenicity index	4.6	3.9	

differentially affects the body weight and the serum cholesterol level in untrained persons differing in the initial values of these indices.

In the group taken as a whole, the mean cholesterol level did not exceed normal values and did not change in adaptation. The circulating triglycerides were also normal, and there was only a tendency to their decrease in adaptation. The average body weight reduction was about 2 kg (from 80.2 to 78.1 kg). Thus, in persons without pronounced disturbances of cholesterol metabolism or appreciable obesity, this mode of adaptation does not affect the lipid metabolism and just moderately reduces the body weight. These results are noteworthy because both humans and animals exposed to stress⁵⁷⁻⁵⁹ develop pronounced hypercholesterolemia observed even with a constant diet. From this viewpoint our adaptation to gradually increasing hypoxia was not apparently stressful for the subjects.

Then we examined a group of 15 subjects with excessive weight and disturbed cholesterol metabolism (Table 13). In this group total cholesterol and circulating triglycerides exceeded the norm, while the cholesterol content in the antiatherogenic HDLP fraction was subnormal; accordingly, the atherogenicity index was elevated to 4.6 (normal values 3 and less).⁶⁰ In this case adaptation decreased the total cholesterol and triglyceride contents and did not change the HDLP cholesterol; as a result, the atherogenicity index decreased to 3.9. It is

noteworthy that the average body weight reduction in this group was greater (from 95.2 to 91.1 kg). It can therefore be supposed that adaptation to hypoxia (which has been experimentally shown to activate cholesterol oxidation to bile acids in the liver) affects the lipid metabolism and the body weight mostly in those cases when these parameters substantially deviate from the norm.

In the aggregate, it can be stated that *in untrained, apparently healthy subjects adaptation to hypoxia increased the tolerance to physical loads, improved the performance and efficiency of circulation and external respiration, and decreased somewhat the body weight in hypercholesterolemic and obese subjects.*

It was also noted that half of the smokers examined dropped the habit. To appreciate these facts, it should be borne in mind that decreased tolerance to physical load, elevated arterial pressure, excess body weight, smoking, and atherogenic dyslipidemia constitute the major risk factors of ischemic heart disease. The observed reduction of these factors can be a point of departure for a special quantitative study of the effects of adaptation to intermittent hypoxia in these respects.

An interesting facet of the problem is the influence of adaptation on the amount of circulating immune complexes (CIC), which are held to be important in atherogenesis⁶¹ and development of IHD.⁶² Thus, examination of a group of 26 enlisted men (yardbirds) experiencing new social conditions, greater physical loads, and hence a certain extent of stress, has revealed markedly higher amounts of CIC (91.0 ± 6.7 U) as compared with a control group of donors of the same sex and age (50.1 ± 4.4 U). This is quite in line with the concept of the aggravating influence of stress on the formation of CIC,⁶³ which is associated with the poststress enhancement of the humoral immune response⁶⁴⁻⁶⁵ and appearance of tissue antigens in the circulation.^{66,67} A course of adaptation resulted in a pronounced decrease in this index (42.2 ± 4.5 U) which then remained close to normal for at least one month after the end of hypoxic exposure (61.4 ± 3.4 U).

There are at least two ways in which the CIC may be involved in the pathogenesis of IHD: interference with the lipoprotein turnover and damage to the vascular endothelium. Thus, the above data can be regarded as a beneficial effect of adaptation to intermittent hypoxia on an important immune mechanism that may under certain conditions aggravate the risk factors of IHD. On the whole, the influence of adaptation on the IHD risk factors undoubtedly deserves further study.

VII. EFFECT OF ADAPTATION TO INTERMITTENT HYPOXIA ON THE COURSE OF NEURASTHENIA AND THE EXTENT OF ARRHYTHMIAS IN NEUROCIRCULATORY DYSTONIA*

Cardiologists and neuropathologists know patients with the neurasthenic syndrome attended by pronounced arrhythmias that, being pathogenetically vague, are often termed idiopathic. On the one hand, direct clinicophysiological studies of the last decades testify that even in paroxysms of such arrhythmias the coronary flow is adequately enhanced, i.e., there is no ischemia.⁶⁸ Moreover, such arrhythmias may progress and even develop into idiopathic heart fibrillation with angiographically intact coronary arteries. On the other hand, quite important in this pathogenetic process are the psychopathic peculiarities of the personality and the occurrence of stressful situations,^{21,69} and this is what points to the neurogenic origin of such arrhythmias (Chapter 2).

All this was put forth as a basis for treating neurasthenias and such arrhythmias with the same set of drugs, namely mild tranquilizers, neuroleptics, and antidepressants.⁷⁰⁻⁷¹ However, this therapy is far from being always efficacious, but most commonly has more or less pronounced side effects altering the patients' behavior.

* Participants in the work: A. A. Lebedev, Ya. I. Kots, V. I. Kornev, V. B. Volovich, I. A. Aleshin, V. S. Grigor'evskikh, R. E. Funin, O. A. Altukhov, and V. Bobylev.

Since adaptation to intermittent hypoxia activates the central stress-limiting systems and concurrently restricts or abolishes the disturbances to cardiac electric stability, it was reasonable to assess its effect on the course of neurasthenia and mentioned arrhythmias. We have examined two groups of patients:

1. Neurasthenic patients with depressive or phobic disorders — 15 persons aged 16 to 37, selected and examined at the Orenburg psychoneurological dispensary
2. Patients with idiopathic (i.e., neurogenic) arrhythmias — 13 persons aged 19 to 41, selected and examined at the therapeutic clinic of the Orenburg Medical Institute.

Before adaptation, the neurasthenic patients displayed pronounced phobic, depressive, and autonomic disorders (cardiophobia, nosophobia, fear of leaving the house alone, fear of crossing the street, anxiety, tearfulness and fatigue, persisting headaches, impaired attention and working ability, sweating, and cold limbs). The 15 patients with a marked syndrome of this kind were specially selected from a large population to assess the effect of adaptation as definitely as possible. They were also subjected to psychological tests (proofreading test, Krepelin's numerical ability test, and Schulte's table-reading test of attention).

Adaptation resulted in substantial improvement of the patients' condition. The effect was most clearly pronounced in the abatement of phobic reactions; the long-sustained fears disappeared or were emotionally deactualized, resulting in normalized behavior and relief of emotional tension in communication. The patients with depressive disorders developed interest for the surroundings, a more favorable attitude to people, and a brighter outlook for the future. By the end of the adaptation course, radical improvement was observed in seven patients and improvement with clearly reduced symptoms in the other eight.

In the group as a whole, the average number of mistakes in the proofreading test decreased from 22 ± 4 to 11 ± 3 , indicating improved attention and concentrating ability. Similar results were obtained in the Krepelin's counting test (initial mean of 48 ± 4 correct additions in 5 min increasing to 62 ± 4 after adaptation) and in the Schulte's test (time needed to find 25 consecutive numbers in the table decreasing from 235 ± 11 s to 194 ± 11 s). In these objective psychological tests for attention and the rate of mental processes, positive shifts were observed in the overwhelming majority of patients: all 15 in the Krepelin's test, and 13 in the proofreading and Schulte's tests; in one case there were no changes in the two latter tests, and there was a single case of some deterioration.

Taking into account the beneficial effect of adaptation on the condition of neurasthenic patients, we then studied its influence on patients with idiopathic nonischemic arrhythmias exhibiting a hypochondriac syndrome. They were complaining of heart intermissions, cardialgias, lack of air, sleep disorders, and were overanxious about their health. In all cases antiarrhythmic drugs were not effective enough. Latent coronary failure or other organic heart diseases were ruled out by standard veloergometric testing, an electrophysiological study with transesophageal cardiac stimulation, ECG in 12 indirect leads, and echoCG. We could therefore be sure that we were dealing with regulatory arrhythmias.

The main means of quantitating these arrhythmias was the 24-h monitoring with a Lenta-MT unit. All 13 patients examined had ventricular extrasystoles and 10 of them had supraventricular extrasystoles as well. By the type of ventricular extrasystoles, six patients belonged to Lown's class 1-2, three to class 3, and four to class 4.

Table 14 lists the cumulative number of extrasystoles in 24 h for each individual case, and demonstrates the effect of adaptation on these rhythm disorders. As can be seen, when the relative changes are averaged, adaptation to intermittent hypoxia decreases the number of both ventricular and supraventricular extrasystoles about threefold. The antiarrhythmic effect is more pronounced in men: ventricular extrasystoles are reduced by $82 \pm 4\%$ ($p < 0.001$) vs. $47 \pm 15\%$ ($p < 0.05$) in women, and the pattern is much the same with

TABLE 14
Results of 24-h ECG Monitoring Before and After Adaptation to Intermittent Hypoxia

Case no. (sex)	Ventricular ES		Supraventricular ES		Sinoatrial block		Group ventricular ES		Beats in 24 h	
	Before	After	%change	Before	After	%change	Before	After	Before	After
1 (F)	11,461	9,214	-20	3	—	-100	—	—	—	—
2 (F)	1	—	-100	100	54	-46	95	12	—	—
3 (F)	27,479	25,332	-8	—	—	—	—	—	83,670	86,951
4 (F)	2,211	1,042	-53	—	—	—	—	—	469	82,458
5 (F)	111	45	-59	2	—	-100	—	—	108	76,864
6 (M)	24,846	14,503	-42	—	—	—	—	—	184	77,167
7 (M)	42	2	-95	40	—	-100	—	—	—	85,596
8 (M)	45	—	-100	69	14	-80	22	—	—	92,328
9 (M)	1,525	504	-67	34	25	-24	—	—	98,926	110,006
10 (M)	881	82	-91	326	42	-87	458	8	—	—
11 (M)	39	12	-69	4,083	2,252	-45	—	—	—	—
12 (M)	428	107	-75	300	15	-96	—	—	—	—
13 (M)	91	19	-79	24	20	-17	—	—	—	—
Mean change				<i>67 ± 8</i>			<i>69 ± 9</i>			
				(<i>p</i> < 0.001)			(<i>p</i> < 0.001)			

supraventricular extrasystoles. When the day and the night periods are analyzed separately, ventricular extrasystole is more affected during the day ($86 \pm 8\%$ in men and $63 \pm 10\%$ in women); the effect on supraventricular extrasystole is about the same in either period. It is noteworthy that adaptation suppresses group extrasystole, which is the most grave form in this contingent: it was completely abolished in 3 out of 5 patients and reduced more than by half in the fourth. There was a single case when despite some reduction of ventricular extrasystole the number of group extrasystoles increased; this turned out to be due to a marked (more than 10-mm) mitral valve prolapse, which appears to be a contraindication against adaptation to hypoxia in the altitude chamber.

Out of three patients with recorded sinoatrial block, such periods completely disappeared in one case and became much less frequent in the other two.

The antiarrhythmic effects of adaptation appear to be rather selective, as there were no appreciable changes in the diurnal heart rate, sinus node function, sinoatrial conduction, etc.

After adaptation, just as with the neurasthenic patients described above, there was marked improvement of the psychic status, and first of all abatement of the hypochondriac syndrome, normalization of sleep, disappearance of cardialgias, and fewer complaints of intermissions and palpitation. This improvement was more pronounced among men. Thus, adaptation to intermittent hypoxia has a definite therapeutic effect in neurasthenia with phobic and depressive phenomena, and a marked antiarrhythmic effect in so-called idiopathic and in essence regulatory arrhythmias.

The latter effect is quite in line with the earlier experimental works on the antiarrhythmic action of adaptation in stress, acute ischemia and reperfusion, infarction, and postinfarction cardiosclerosis.¹⁹ Most probably it is due to the adaptive activation of the stress-limiting systems — opioidergic, serotonergic, antioxidant ones^{11,45} — whose metabolites act as antiarrhythmics.⁷² Since in animals adaptation to intermittent hypoxia is also known to reduce the postinfarct scar,¹⁹ the body of data obtained makes promising the use of such adaptation for abolishing arrhythmias in and rehabilitating patients with certain forms of postinfarction cardiosclerosis.

By and large, these data would suffice to demonstrate the potential of adaptation to hypoxia for prevention and treatment of some heart diseases, and, conforming to the framework of this book, we could have restricted ourselves to that. However, the earlier experimental facts that such adaptation causes profound changes in the immune system, suppresses the development of allergic arthritis in animals, and decreases the amount of immune complexes in the blood prompted us to use it in treating some allergic diseases. The positive effects obtained are considered in the next section and, in our opinion, testify that the potentialities of adaptation to intermittent hypoxia go far beyond the frame of cardiology.

VIII. EFFECT OF ADAPTATION TO INTERMITTENT HYPOXIA ON THE INDICES OF THE IMMUNE SYSTEM AND ON THE COURSE OF SOME ALLERGIC DISEASES*

Examination and adaptation to intermittent hypoxia with therapeutic purposes has been carried out with three groups of patients.

The first group was children (6 to 14 years old) with atopic bronchial asthma; the duration of disease was 2 to 6 years, and drug therapy had been at best only briefly effective.

* Participants in the work: M. N. Volyanik, V. K. Bannikov, A. I. Smolyagin, S. N. Afonina, N. M. Lifshits, M. A. Frolova, I. N. Chainikova, V. K. Fillipov, I. Yu. Kil'divatov, A. A. Nikonorov, O. P. Pavlovskaya, and O. G. Kislova.

Adaptation to hypoxia in the *Ural-1* multiseater altitude chamber was carried out according to a modified technique⁷³ successfully used for several years.¹⁸ The adaptation course consisted of 20 to 25 seances; in the first 5, the patients were gradually "taken up" to 3.5 km above sea level. The "ascent" and "descent" rate in each seance was 2 to 3 m/s and each exposure lasted 1 h.

The second group was adult patients with allergic dermatoses. The total number was 21 (12 men and 9 women); the duration of diseases was 4 to 30 years; in all cases drug therapy was poorly effective.

The third group consisted of six women aged 30 to 40, with autoimmune thyroiditis (Hashimoto's disease) of 1- to 10-year duration; in all cases conventional treatment had poor results.

The adults in the second and third groups were adapted similarly to the children, with one essential difference that each hypoxic exposure lasted 3.5 h.

All patients before and after adaptation were examined for the major indices of the immune status: the number of B and T lymphocytes in rosette tests,⁷⁴ the serum contents of C3 (complement factor) and immunoglobulins by radial immunodiffusion,⁷⁵ and CIC by polyethylene glycol precipitation.⁷⁶ For patients with thyroiditis, the titer of autoantibodies to thyroid water-salt extracts was determined by passive hemagglutination according to Boyd.⁷⁷ Patients with allergic dermatoses were tested for the intensity of cell response in the phytohemagglutinin test and histamine induction of T suppressors.⁷⁸ Asthmatic children were also tested for several indices of neurohumoral regulation: plasma 11-oxy corticosterone content⁷⁹ including its free and protein-bound fractions,⁸⁰ blood histamine⁸¹ and serotonin,⁸² and catecholamine excretion with urine.⁸³

It has been found that children with atopic bronchial asthma display no pronounced deviations from the norm in the total amount of lymphocytes and circulating T cells, though the relative amount of B cells is somewhat increased. At the same time, the serum IgG and IgM contents are appreciably reduced (36 and 17%, respectively), while the CIC are elevated 2.5-fold. This suggests that such disbalance between the higher B count and the lower Ig content reflects not just a functional failure of the B system, but rather a pathogenetic link of the disease associated with enhanced formation of immune complexes which are important triggers of allergic disorders in this kind of pathology.⁸⁴

Adaptation of the asthmatic children to intermittent hypoxia caused some further increase in the relative content of B lymphocytes, but, most importantly, completely normalized the serum Ig and CIC levels. It is noteworthy that the decline in CIC had a corollary in the enhanced C3 content, which is likely to reflect the reinforced organismic defense against immune-complex pathology since activated C3 is known to disperse the CIC.⁸⁵ Of especial practical importance is the fact that such normalization of the humoral immune indices was observed in an overwhelming majority of children with bronchial asthma: CIC reduction in 86% of cases, enhanced C3 in 91%, restored Ig in 70% (IgA) to 83% (IgM).

This beneficial effect of adaptation on the immune system in bronchial asthma appears to be associated with certain changes in neurohumoral regulation. Indeed, there was an increase in the blood 11-oxy corticosterone content and especially in its free physiologically active fraction, an increase in the sympathoadrenal activity revealed as enhanced catecholamine excretion with urine (epinephrine by 69%, norepinephrine by 117%), and a substantial decrease in blood histamine, which is a major mediator of allergic reactions.⁸⁶

All this makes understandable why the curative effect of adaptation, which is due to natural regulatory readjustment of the immune system, is incomparably more stable than that of many drug-based approaches, and obviates the use of such extreme and often dangerous means as stress hormones.⁸⁷

In the second group, 21 patients with allergic dermatoses (18 with atopic neurodermatitis and 3 with true eczema) were evaluated with respect to their clinical status, the above-

TABLE 15
Effect of Adaptation to Intermittent Hypoxia on the Indices of
Cell and Humoral Immune Status of Patients with Allergic
Dermatoses

Indices	Healthy controls	Patients	
		Before	After
Leukocytes (count per mm ³)	6100 ± 205	6430 ± 144	6600 ± 151
Lymphocytes (per mm ³)	1719 ± 41	1736 ± 65	1718 ± 62
(% leuk.)	28.2 ± 1.4	27.0 ± 0.7	27.3 ± 0.9
T cells (per mm ³)	1258 ± 43	1128 ± 60	984 ± 77 ^a
(% lymph.)	69.9 ± 1.5	64.8 ± 2.7	56.0 ± 3.9 ^a
B cells (per mm ³)	337 ± 27	425 ± 53	409 ± 51
(% lymph.)	19.4 ± 2.3	24.5 ± 2.8	20.9 ± 2.2
Phytohemagglutinin test (mm)			
PHA	26.1 ± 1.7	15.5 ± 2.2 ^a	15.9 ± 2.8 ^a
PHA + histamine	27.6 ± 1.9	5.2 ± 1.1 ^a	13.7 ± 1.9 ^a
Difference	+1.5 ± 0.4	-10.3 ± 2.3 ^a	-2.6 ± 2.6
Immunoglobulins (mg %)			
A	254 ± 19	109 ± 12 ^a	233 ± 40
M	151 ± 14	87 ± 11 ^a	127 ± 20
G	1847 ± 287	991 ± 59 ^a	1658 ± 183
E	—	460 ± 106	339 ± 123
CIC (U)	50 ± 4	68 ± 7	33 ± 4
Histamine (ng/ml)	40 ± 3	75 ± 3 ^a	37 ± 7

^a $p < 0.05$.

mentioned immunological parameters, and blood histamine. The clinical evaluation included the total afflicted skin area, the area of continuous lesion (percent), and the extent of pruritus on a five-point scale.

Directly after the course of adaptation to intermittent hypoxia in the altitude chamber, improvement was observed in 9.6% of patients, marked improvement in 80.9%, and clinical convalescence in 9.5%. The total afflicted area was reduced from $44 \pm 6\%$ to $16 \pm 5\%$, continuous lesion area from $29 \pm 6\%$ to $8 \pm 5\%$, and pruritus from 3.7 to 0.2 points on the average. Concurrently, the immunological indices determined in ten patients were also normalized as shown in Table 15: the contents of IgA, G, and M in the serum increased while that of IgE decreased, and CIC fell more than by half. The T suppressors became less sensitive to histamine as evidenced by the considerably smaller effect of histamine in the intracutaneous phytohemagglutinin test, and the blood histamine became normal.

There were no changes in the leukocyte count, absolute and relative number of lymphocytes or T and B populations, i.e., the adaptation had no immunodepressive effect.

At 1 month after the course, 18 patients could be re-examined; clinical convalescence was documented for 50%, marked improvement for 39%, and only 11% showed re-exacerbation by that moment. The total afflicted skin area in 1 month was $14 \pm 5\%$, and pruritus (1.0 point) still remained much abated.

In assessing the positive result of the adaptational treatment of allergic dermatoses, attention should be paid to two issues.

1. In allergic dermatoses, just as in the allergically originating bronchial asthma, besides other alterations in the immune system there is a substantial rise in the circulating immune complexes, and in both cases this is abolished with adaptation.
2. Treatment of allergic dermatoses with drugs — tranquilizers,⁸⁹ antihistamine agents,⁹⁰ vitamins,⁹¹ immunomodulators^{92,93} — in many cases has no appreciable result. This

has led to the use of agents that certainly have adverse side effects, such as glucocorticoids,^{94,95} or of invasive techniques like hemosorption⁹⁶ and plasmapheresis,⁹⁷ which are fraught with complications.^{98,99} Much better results are obtained with mountain-climate therapy,^{100,101} which is also safe enough, but for most patients this inevitably involves quite long and repeated journeys. Adaptation to intermittent hypoxia in the altitude chamber is devoid of all these shortcomings, and is an efficient and convenient means of treating this pathology. To make a note, there is much in evidence that the duration of the course ought to be extended.

In the third group, the six patients with case histories of 1 to 10 years initially had complaints fitting into the picture of a pronounced autonomic asthenic syndrome. Autoimmune thyroiditis was diagnosed after immunological examination that revealed high titers of antithyroid antibodies in the blood (1:640 to 1:24,800).

After adaptation to intermittent hypoxia, the patients' condition improved and the high erythrocyte sedimentation rate was attenuated.

Quite demonstrative results were obtained in the immunological examination. Before adaptation, the patients with autoimmune thyroiditis had lower counts of blood lymphocytes, mainly at the expense of T cells; the number of B cells did not appreciably change so that their relative content increased. Such changes in the lymphocyte subpopulations may reflect not only the peculiarities of the disease, but also those of the previous treatment, which included (not less than a year before) short courses of glucocorticoid hormones. However, the most significant alterations were observed in the humoral immune status: a twofold increase in CIC with a high level of antithyroid antibodies and lowered content of IgA and IgG.

Adaptation to hypoxia did not normalize the lymphocyte composition, causing some further decline in the portion of T cells, but markedly decreased the contents of CIC (1.6-fold) and antithyroid autoantibodies (titers from 0 to 1:1,280), and restored to normal the IgA and IgG levels. Though it is premature to claim a definite therapeutic effect of adaptation to intermittent hypoxia in Hashimoto's disease, the objective changes in the immune status are clearly much the same as in the allergic diseases considered above.

Thus, formation of a structural trace in the immune system in the course of adaptation to intermittent hypoxia, manifesting itself as an increasing portion of B cells and some reduction of T cells, favors normalization of the humoral defense in allergic (bronchial asthma and eczema) and autoimmune (thyroiditis) diseases. Such adaptation also markedly attenuates, through a mechanism that is still to be elucidated, the amount of circulating immune complexes in these disorders.

IX. PROSPECTS OF THE USE OF ADAPTATION TO INTERMITTENT HYPOXIA

We have seen that adaptation to hypoxia alleviates the detrained state, reduces some IHD risk factors, and hence may be used as an efficient means of preparing the people burdened with such risk factors to physical exercise, i.e., as an element of combined prevention of IHD. Adaptation to intermittent hypoxia is a promising approach to drug-free prevention and therapy in cardiology, neuropathology, pediatrics, and allergology. Still, collation of the positive clinical results with the experimental data gives rise to an impression that the potentialities of this approach are far greater than the present-day achievements.

1. With healthy individuals, adaptation to intermittent hypoxia should be used not just to prepare them for frequent or prolonged stays at high altitudes, flight work, etc., but first and foremost to enhance their resistance to stress, in particular occupational

stress. Adaptation is also a practical means of rehabilitating people prone to stress-linked diseases. With the power of modern analytical methods, such works will also contribute much to elucidation of the role of central and local stress-limiting systems in the adaptation of the organism to the environment.

2. No less important is the influence of adaptation to hypoxia on the circulation and neuroendocrine regulation in selected contingents with hereditary hazards and manifest risk factors of IHD and hypertension. Attended with caution, adaptation may be used even in the postinfarction stage to prevent recurrences of infarction, arrhythmias, and impairment of cardiac contractility, i.e., to attain the goals already documented in the experiment.
3. There seems to be much promise in the use of adaptation to hypoxia to treat the major endogenous psychoses, in particular some forms of schizophrenia. In this context, a marked intrinsic similarity must be accentuated between the insulin-shock treatment and adaptation to hypoxia. Indeed, with increasing doses of insulin the brain has to adapt to a deficiency of the main energy source, glucose, whereas with repeated hypoxia it adapts to a deficiency of the ultimate electron acceptor, oxygen. At the same time it is obvious that adaptation to hypoxia is incomparably more sparing than insulin shocks or massive doses of psychotropic drugs used nowadays to treat schizophrenia. Provided strict observance of the contraindications mainly related to the state of circulation, and rational use of sedatives during the actual hypoxic exposure, this method appears undebatably safe, and assessment of its efficacy highly expedient.
4. Another aspect of the influence of adaptation on the higher divisions of the human nervous system is that it promotes accumulation of large amounts of opioid peptides in the adrenals and mobilization of this reserve in stress, and prevents not only stress damage, but also the decline in the opioid peptide content in the brain. Since it is known that the craving for opiates may be related to their endogenous deficit, a question of considerable practical import arises as to how adaptation to intermittent hypoxia would affect the addiction to morphine.
5. The most currently tangible line of development is further and expanded use of adaptation to hypoxia for treating allergic and autoimmune disorders such as rheumatic arthritis, autoimmune hemolytic anemias, autoimmune juvenile diabetes, autoimmune nephropathies, myasthenias, and others.
6. With certain reserve, another trend stems from a number of experimental observations that adaptation of animals in the altitude chamber impedes growth of tumors.¹⁰³⁻¹⁰⁶ The most recent data¹⁰⁷ show that this line of research has at least two different aspects: first, the impeding effect of adaptation on tumor growth through the antitumor immunity system, and second, the application of adaptation to hypoxia in combined tumor treatment with drugs, radiation, and surgery. In this case advantage can be taken, among other things, of the ability of adaptation to enhance the organismic resistance to surgical stress and ionizing radiation¹⁰⁸ as well as to chemical agents; thus, adaptation may be helpful at the rehabilitation stage after the radical modern measures of tumor treatment.
7. Finally and unavoidably, notwithstanding all the commendable features of the *Ural-1* chamber, the novel and differentiated applications of adaptation to hypoxia would call for new specialized and optimized designs of altitude chambers.

All new trends have one thing in common: their development is unpredictable. Therefore, today it is hardly possible and really needless to guess which of the above-listed aspects will yield the most notable results. Obviously, these and other potentialities of adaptational therapy can only be actualized provided faithful participation of clinicians and high standards of general and specialized examination. In any case, it must be underscored again that this

painstaking work on the development of the novel method of drug-free therapy and prophylaxis follows the same path that the organism usually takes itself by adapting to the milieu and in just this way protecting itself from damage. In other words, adaptation is the cornerstone of the natural prevention of disease. Relying upon the general principle "do as Nature does", it may in future be possible to check the inflationary dependence of mankind on assorted drugs, and to learn to cure some now refractory maladies.

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Chapter 8

CONCLUSION

In this book, with the heart as a vivid example, we have considered three types of mechanisms that have not been discussed heretofore and that help the organism to prevent or abolish stress-induced, ischemic, reperfusion, allergic, chemical, and even radiation damage.

The first type of such defense forms as the organism adapts to repetitive action of one particular environmental factor, for instance, hypoxia. As it does so, the adaptation has its material basis in a systemic structural trace; the latter has quite ramified architectonics, with elements ensuring enhanced resistance not to a single factor, but to many environmental factors. The scheme in Figure 1 demonstrates this cross-protective effect of adaptation to intermittent hypoxia, which we could have seen to be quite useful not only in experimental prophylaxis and therapy, but also in the treatment of several rather diverse human diseases. In our opinion, such cross protection is the most important principle of drug-free prophylaxis and therapy, and shows much promise, in particular in preventive cardiology.

The second type of defense, which we have considered in Chapters 3 through 5, relates to the concept of stress-limiting systems; these systems are coupled with the stress reaction and check it at both central and cellular levels, preventing thereby the stress-induced damage. In this mechanism, the organism adapts not to any particular and specific factor, but rather to unavoidable physiologically stressful situations. A course of such brief and mild enough, noninjurious exposures to stress gradually enhances the power or the efficiency of central and local stress-limiting systems: from the opioidergic and the GABA-ergic ones in the brain to the prostaglandin and the antioxidant ones in the cells and organs.

Thus, in this case the systemic structural trace of such adaptation also proves rather ramified, and there is also a broad cross-protective effect manifesting itself as not just enhanced resistance to severe emotional pain stress (EPS) or other kinds of stress, but also protection of the heart from ischemic and reperfusion damage and arrhythmias, and of the whole organism from direct chemical or radiation injury, etc. As depicted in Figure 2, in the organism adapted to totally nonspecific hard situations, the greater physiological power of the panoply of stress-limiting systems really protects it against stress-linked injury in exposure to diverse external influences. This attempt at a physiological interpretation of the "university of hard knocks" still covers only part of the issue, since rigorous experiments of this decade have shown that activation of the stress-limiting systems, besides protecting the animals' internal organs from damage, profoundly alters their behavior in a biologically advantageous direction. Such data are available for the GABA-ergic,¹ the opioidergic,² the serotonergic,³ the antioxidant,⁴ and other systems.

The contents of this book show that there are at least four ways in which the stress-limiting systems can be activated with preventive purposes in cardiology and other fields of clinical medicine.

1. Repetitive dosed exposure to moderate stress. As shown recently, this can be achieved not only by immobilization or EPS, but equally well by means quite applicable to man, such as electroacupuncture or exposure to cold. Such treatment is consistently accompanied with activation of the opioidergic and other stress-limiting systems; an experimental course of transauricular electroacupuncture prevents the stress-induced drop in the cardiac fibrillation threshold and abates the ischemic arrhythmias. Thus, the contemporary physiotherapeutic and reflexotherapeutic means offer a practicable way of enhancing the efficiency of the stress-limiting systems and consequently the resistance of the heart to stress, ischemic, and other kinds of damage.

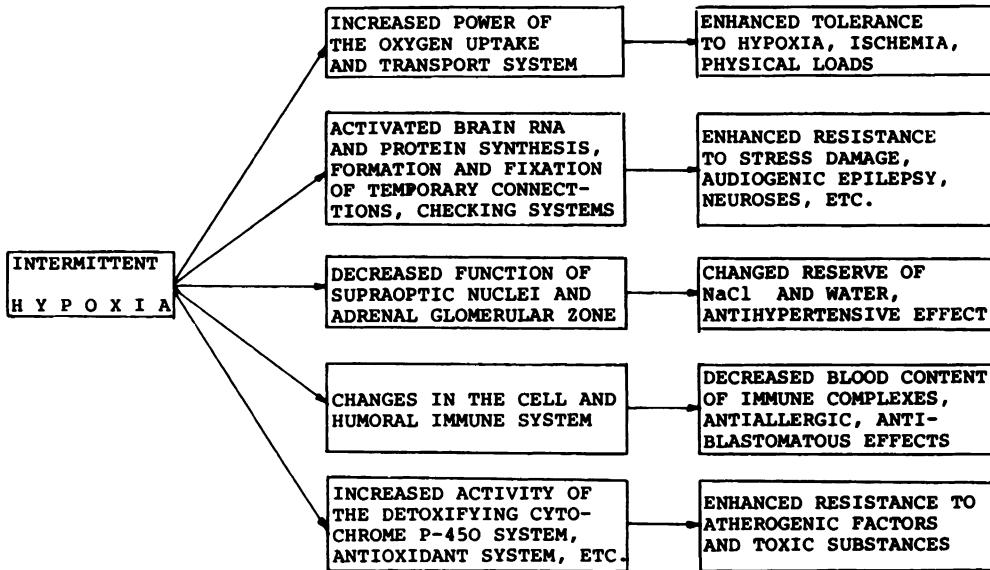


FIGURE 1. The systemic structural trace and the protective effects of long-term adaptation to hypoxia.

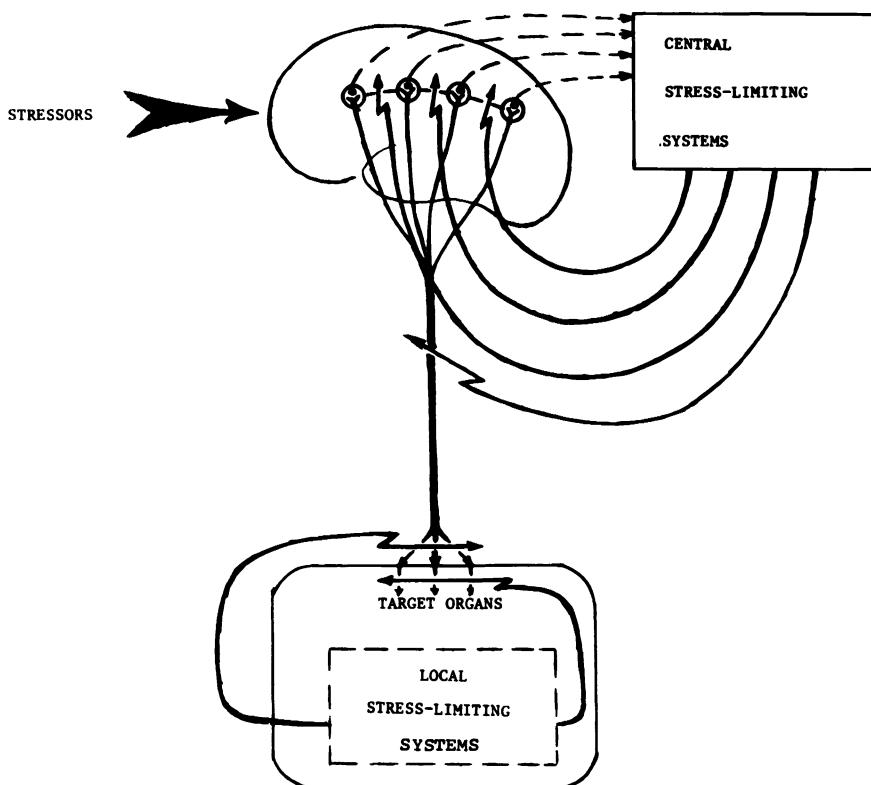


FIGURE 2. Stress-linked damage is prevented by central and local stress-limiting systems.

2. Adaptation to moderate exposure to various environmental factors. Since a stress reaction is evoked by the most diverse external factors acting on the organism, the stress-limiting systems are also naturally activated. Thus we have seen that in adaptation to intermittent high altitude hypoxia there are increases in the opioid peptide content in the adrenals, serotonin in the brain, antioxidant enzymes in the cardiac muscle, etc. These and other important adaptive changes have allowed the use of adaptation to intermittent hypoxia in the altitude chamber to reduce the risk factors of ischemic heart disease, to abolish grave forms of neurasthenia and the so-called idiopathic (neurogenic) arrhythmias.
3. Use of pharmacological activators of stress-limiting systems, which enhance the content of their mediators or the activity of their receptors. Such effects on the GABA-ergic system are exhibited by sodium valproate, which raises the blood and brain GABA levels, and by agonists of benzodiazepine receptors potentiating the GABA effects; stimulatory for the serotonergic system are opioid peptides and serotonin precursors, and so on.
4. Administration of natural mediators of the stress-limiting systems, or synthetic analogs thereof, to directly make up for the insufficiency of these systems. Examples of these are GABA, GHBA, opioid peptides and their synthetic analogs, serotonin, α -tocopherol, synthetic antioxidants, coenzyme Q, and adenosine derivatives.

The third type of defense built up by the organism in the course of adaptation (to stress and probably other factors) reveals itself in that the cell structures and systems become "hardier", i.e., can better withstand the action of the natural degrading agents involved in autolysis. A putative mechanism of this stabilization phenomenon has been considered in Chapter 6 (Figure 13), and implies an important role of "stress proteins" discovered in the last decade. Regardless of how valid this hypothesis, the phenomenon as such is an irrefutable fact. *In vitro*, autolysis and inactivation of SR membranes, mitochondria, and creatine phosphokinase are markedly retarded in preparations from stress-adapted animals. *In vivo*, prior adaptation to stress diminishes by 40% the infarct size despite the same ischemic zone in coronary ligation, i.e., enables the cells to withstand ischemia. Of course, to make practical use of such mechanisms is a quite appealing, albeit in many aspects debatable, goal.

By and large, the drug-free adaptational protection of the organism, and first of all the heart, follows the most natural way that the organism normally takes itself as it adapts to the environment to avoid damage. Clearly, active control over this process, rational dosing, and combination of the factors to which adaptation takes place will make such protection more efficient. At the same time the knowledge of the basic molecular mechanisms of adaptation will allow the use of metabolites that naturally take part in this process, and thus speed up the development of unorthodox approaches to pharmacological protection of the heart. In the end, it should be stressed that all this is but one example from the now rapidly emerging field of *adaptational medicine*. This is indeed the medicine of the nearest future.

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