Development of Cardiac Sensitivity to Oxygen Deficiency: Comparative and Ontogenetic Aspects

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I. Introduction

Clinical-epidemiological studies have shown that the risk factors of serious cardiovascular diseases, such as atherosclerosis and ischemic heart disease, are present during the early phases of ontogenetic development (57) (Fig. 1). Some of them, including excessive food intake and an increased level of cholesterol, operate directly...
after birth, whereas genetic factors are present even before birth. Thus atherosclerosis and ischemic heart disease can no longer be considered the diseases of the fifth and higher decades of life, but their origin and consequences may be essentially influenced by risk factors acting already during development. It follows that experimental studies of the pathogenic mechanisms of these disturbances must shift to the early ontogenetic periods. Accordingly, it is not surprising that the interest of theoretical and clinical cardiologists in the developmental approach keeps increasing. The advances of molecular biology (226, 227) as well as the developing possibilities of prenatal cardiology (221) have substantially accelerated this trend.

The most frequent (and hence the most widely studied) cardiovascular diseases of modern times undoubtedly include hypoxic states. They originate as a result of disproportion between the amount of oxygen supplied to the cardiac cell and the amount actually required by the cell. The degree of injury depends, however, not only on the intensity and duration of the hypoxic stimulus, but also on the level of cardiac tolerance to oxygen deprivation. In recent years, the attention of cardiac research laboratories has concentrated on the theoretical basis of rational prevention and therapy of cardiovascular problems, such as myocardial ischemia and chronic hypoxia, leading to the development of cor pulmonale. Although abundant data are available concerning the effects of ischemia and hypoxia on the adult myocardium, very little is known about the influence of oxygen deprivation on the developing cardiac muscle. Furthermore, the available literature on ontogeny is scarce and rather controversial. Whereas up to 1987 there had been a consensus that the immature heart was more resistant to oxygen deprivation than the adult one, possibly as a consequence of its greater capability for anaerobic glycolysis (for review, see Ref. 203), in 1987 several publications provided some indications that the immature heart might in fact be more susceptible to ischemic injury than the adult one (e.g., Refs. 175, 250). Unfortunately, the ontogenetic conclusions are often based on a simple comparison of the adult myocardium with a randomly selected and poorly defined developmental stage (e.g., “fetal,” “newborn”). The possible developmental changes between selected (and not exactly defined) points thus remain undetermined (152).

Although it is impossible to agree fully with the statement that the ontogenetic development is a replication of phylogeny, comparative studies (more correct than “phylogenetic,” since the researcher is comparing not the whole evolutionary range but only some classes, i.e., a synecdochic approach) have contributed significantly to our understanding of the function of the vertebrates cardiovascular system (33, 181). From the point of view of developmental cardiology, the advantage of comparative studies lies in the possibility of studying selected developmental periods as stable situations, which is impossible for the rapidly changing mammalian ontogeny. Better understanding of the development of both sides of the essential equation of cardiac homeostasis, i.e., oxygen supply = oxygen consumption, may serve as a typical example; it would be unthinkable without significant contribution of a comparative approach.

Therefore, in this review, our intention is to deal with comparative and ontogenetic aspects of the development of systems responsible for myocardial oxygen supply and myocardial oxygen consumption and to stress their role in the variations in cardiac sensitivity to oxygen deprivation.
Particular attention is paid to the possible endogenous protection of the immature heart against oxygen deprivation (adaptation to chronic hypoxia, preconditioning) as well as to the developmental limitations of exogenous protection provided by pharmacological interventions. The clinical implications of hypoxic congenital heart disease, intraoperative exposure of the immature heart to ischemia, cardioplegia, and possible risks for the clinical use of inotropic drugs in obstetrics and pediatric cardiology are also emphasized.

II. CARDIAC HYPOXIA AND ISCHEMIA

A. Definition

As stated in section I, myocardial hypoxia is the result of disproportion between oxygen supply and demand. Because of the high coronary arteriovenous difference, the myocardium is not able to bring about a substantial improvement in the oxygen supply by increased extraction of oxygen from the blood, and thus the only way of meeting the high demands is through an increased blood supply. Oxygen consumption depends on the heart rate, contractile performance, and tension of the ventricular wall, as a result of the pressure-volume relationship in the ventricle itself (78). Theoretically, any of the known mechanisms leading to tissue hypoxia can be responsible for a reduced oxygen supply in the myocardium, but the most common causes are undoubtedly 1) ischemic hypoxia (often described as “cardiac ischemia”) induced by reduction or interruption of the coronary blood flow and 2) systemic (hypoxic) hypoxia (“cardiac hypoxia”) characterized by a drop in PO2 in the arterial blood but adequate perfusion. Anoxia is the absence of oxygen supply despite adequate perfusion. For the sake of completeness, we could add 3) anemic hypoxia in which the arterial PO2 is normal but the oxygen transport capacity of the blood is decreased and 4) histotoxic hypoxia resulting from reduced intracellular utilization of oxygen in the presence of adequate saturation and an adequate blood flow (e.g., inhibition of oxidative enzymes in cyanide poisoning). The most frequent causes of high oxygen consumption are increased physical activity, mental stress, or administration of a substance with a positive inotropic and chronotropic effect. In healthy subjects, these high oxygen requirements are adequately met by an increase in the coronary blood flow.

It should be emphasized that the terms hypoxia and ischemia are unfortunately often used interchangeably in the literature despite the fact that the consequences of the two mechanisms at the cellular level are very different (153). In ischemia, there is not only a drop in the supply of oxygen and substrates, but also a significant reduction in the clearance of metabolites, in particular of lactic acid and hydrogen ions; the intracellular pH falls rapidly as the acid products of glycolysis accumulate. In contrast, in cardiac hypoxia, perfusion results in the washing out of the acid products of glycolysis, thereby retarding the rate of development of acidosis (197). Systemic hypoxia is usually a generalized phenomenon diffusely involving the whole myocardium, whereas ischemia is confined to the area supplied by the affected coronary artery. Ischemic hypoxia is clinically manifested primarily in ischemic heart disease and its acute form, myocardial infarction, whereas systemic hypoxia is associated with chronic cor pulmonale of varying origin, cyanosis due to a hypoxic congenital heart disease, and changes induced in the cardiopulmonary system by a decrease in barometric pressure at high altitudes. In two cases, however, systemic hypoxia can be qualified as physiological: 1) the fetal myocardium that is adapted to hypoxia corresponding to an altitude of 8,000 m (“Mount Everest in utero”) (202) and 2) the myocardium of subjects living permanently at high altitudes. In both situations, the myocardium is significantly more resistant to acute oxygen deficiency, but in populations living in lowlands, this property is lost soon after birth (72, 137).

Unfortunately, there is still no existing experimental model that adequately reproduces all the functional, structural, and metabolic changes that are characteristic for hypoxic states of the human cardiopulmonary system. The basic problem is that in most models, we are working with an intact animal in which the systems responsible for the supply and consumption of oxygen are not affected by pathological process. Furthermore, mainly for the methodological reasons, not all experimental models of adult hearts are applicable to the immature myocardium. The most frequently used experimental models of cardiac hypoxia and ischemia are summarized in Table 1.

B. Early Consequences of Oxygen Deprivation

With a reduction in oxygen supply, the adult as well as immature myocardium switches from the aerobic to the

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<th>Table 1. The most frequently used experimental models of cardiac hypoxia and ischemia</th>
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<td><strong>In Vitro</strong></td>
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<td>Hypoxia (↓ PO2 in medium)</td>
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<td>Isolated perfused heart</td>
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<td>Isolated papillary muscle</td>
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<td>Isolated myocytes</td>
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<td>Ischemia (↓ coronary flow)</td>
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↓, Decrease.
anaerobic mode (for review, see Refs. 90, 144). The capacity of the myocytes to generate energy in the form of ATP and creatine phosphate becomes severely depleted, to such an extent (only 3 mol of ATP are produced for every mole of glucose instead of 38 mol that are generated during oxidative metabolism) that the high-energy stores become rapidly depleted (196). The heart ceases to contract within seconds from the onset of oxygen deprivation. However, the precise mechanism of the impairment of the ventricular systolic function has not been defined. The possible etiology includes the ability of hydrogen ions to displace calcium ions from their binding sites on the myofibrils and the failure of calcium transients. Thus the actin-myosin interaction is impaired and contractility is reduced (29). Despite the fact that lack of oxygen depletes ATP from the myocardium, impairment of function is not closely related to depression of overall ATP content, which declines more slowly and is associated with progressive intracellular hypoxia and acidosis (119). Potassium ions migrate from the inside to the outside of the muscle cells, and sodium ions move in the opposite direction. These are important changes, since extracellular accumulation of potassium ion has a profound effect on the transmembrane potential difference, and intracellular accumulation of sodium ions leads to myocardial swelling. Interestingly, the form of oxygen deprivation may modify the events; for example, anaerobic glycolysis is activated and persistent in hypoxic preparations but is depressed to zero in low-flow ischemic preparations.

Soon after the onset of oxygen deprivation, cytosolic calcium increases, leading to an intracellular calcium overload (for review, see Refs. 58, 144). This multifactorial event is of both intracellular and extracellular origin. The extracellular component probably involves the entry of a small amount of calcium in exchange for sodium. The major component is, however, of intracellular origin; mechanisms that are involved include enhanced calcium release from the sarcoplasmic reticulum (possibly because of damage caused by free radical production) (216), failure of the calcium uptake by sarcoplasmic reticulum (because of energy depletion), failure of ATP-dependent sarcolemmal calcium pump responsible for extruding calcium ions across the sarcolemma, and proton-driven efflux of calcium from the mitochondria. As the duration of the hypoxic/ischemic episode progresses, the sarcolemma may become leaky, possibly because of free radical formation and the effect of these newly formed radicals on the architecture of the membrane-located phospholipids. The consequences of this rise in cytosolic calcium include activation of lysosomal proteases and phospholipases, resulting in ultrastructural injury; stimulation of oxygen radical production; activation of latent calcium-dependent ATPases, which hastens depletion of residual ATP reserves; calcium accumulation in mitochondria leading to a defect in energy production; and calcium accumulation in the cytosol in the vicinity of the contractile proteins, which contribute to the raised end-diastolic resting tension (for review, see Refs. 46, 58, 144, 151).

Energy depletion, oxygen radical accumulation, and loss of calcium homeostasis, although major contributors to the cascade of events induced by reduction in oxygen supply, have to be coupled with the consequences of the loss of osmotic control that develops at the same time (144). The accumulation of intracellular substances, including lactate, phosphate, protons, and ammonium ions, presents the myocytes with an “osmotic shock” at a time when the sarcolemma is becoming increasingly fragile. Membrane disruption is the inevitable consequence (90).

C. Reperfusion Injury, Stunning, and Hibernation

All the changes described in section 2B are time dependent. Transient reversible ischemia followed by reperfusion can result in increased production of superoxide radicals (243); they attack proteins and polyunsaturated fatty acids, causing enzyme inactivation and lipid peroxidation, respectively. In reversible ischemia, the intensity of this damage is not sufficient to cause cell death but is sufficient to produce dysfunction of some key cell organelles (e.g., sarcolemma and sarcoplasmic reticulum). This would result in impaired calcium homeostasis: increase of free cytosolic calcium and excitation-contraction uncoupling. The ultimate consequence is a reversible depression of contractility (21). In contrast, reperfusion after prolonged periods of ischemia, at a time when the energy reserves are depleted, the cytosolic calcium level has risen substantially, osmotic equilibrium has been disturbed, and the natural pool of oxygen radical scavengers is depleted, can hasten the death of those cells that were already injured but are potentially salvageable (144).

As stated above, short-lasting myocardial ischemia followed by reperfusion induces contractile abnormalities. This “postischemic stunning” is defined as a mechanical dysfunction that persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or near-normal coronary flow (for review, see Refs. 23, 69). The factors responsible for myocardial stunning consist of two components: a component that develops during ischemia and another component that develops after reperfusion. Myocardial stunning is thus probably a multifactorial process that involves 1) abnormalities of calcium homeostasis occurring during ischemia and reperfusion and 2) generation of oxygen-derived free radicals upon reperfusion. From the effects of antioxidants in models of myocardial stunning, the reperfusion injury component appears to be greater than the ischemic injury component (21). These facts support the important concept (214) that the severity of the reperfusion injury component of myocardial stunning is propor-
tional to the severity of the ischemic injury component. Accordingly, any intervention that attenuates the severity of ischemic injury will also, indirectly, attenuate the severity of the subsequent reperfusion injury.

Myocardial stunning should be distinguished from the concept of “hibernating myocardium.” This can be defined as a persistent (for at least several hours) contractile dysfunction that is associated with reduced coronary flow but preserved myocardial viability (22, 70, 76). This phenomenon is teleologically postulated to be an adaptive response of the heart to low flow, whereby oxygen demand is downregulated to the point that the reduced oxygen supply can be tolerated for an extended period of time without cell death and without clinical or metabolic evidence of ischemia. Once coronary flow is restored, the dysfunction is completely reversed. Thus stunning and hibernation have one thing in common; in both cases, the ventricular dysfunction is reversible. The major difference is that blood flow is normal or near normal in stunned myocardium, whereas it is reduced in hibernating myocardium (22).

III. DEVELOPMENT OF OXYGEN AND ENERGY SUPPLY

As mentioned in section I, the degree of myocardial injury depends not only on the intensity and duration of hypoxic (ischemic) stimulus, but also on the degree of cardiac sensitivity to oxygen deficiency. This variable is determined by the relationship between myocardial oxygen supply and demand, i.e., myocardial blood flow and the oxygen-carrying capacity of blood, on the one hand, and the functional state of cardiac muscle (level of contractile function, systolic wall tension, heart rate, and external work) and basal metabolism, on the other hand (2, 3). Because most of these determinants change significantly during phylogenetic and ontogenetic development, it is not surprising that significant developmental changes also underlie their common consequence, cardiac resistance to oxygen deprivation (33). It may, therefore, be of interest to analyze how the developmental changes in oxygen-supplying and oxygen-consuming systems influence the changes in cardiac sensitivity to hypoxia or ischemia. From this point of view, two evolutionary situations seem to be most important: the transition from poikilothermy to homeothermy in adult vertebrates and the perinatal period in homeotherms.

The comparison of experimental and clinical ontogenetic studies is, however, difficult, to some extent as a consequence of the inconsistent terminology (203). The absence of commonly agreed definitions for individual developmental periods (e.g., newborn, neonate, infant, and immature) has undoubtedly hampered clarification in this field, particularly in animal studies. Because the majority of the experimental studies referred to in this survey have been carried out in the rat, we will, for the sake of consistency, use the following definitions: neonate (newborn), <1 wk old; suckling, up to 2 wk old; weaning, 2–4 wk old; sexual maturation, 4–8 wk old; adult, 8 wk old and beyond (8). Results from species other than rat are also discussed, and we will adopt the definitions used by the respective authors.

A. Development of Myocardial Blood Supply

1. Comparative aspects

Performance of the heart as a pump must be precisely adapted to the oxygen consumption of the total active body mass. As a result, the heart size in different species of vertebrates, expressed as a ratio of heart weight to body weight (i.e., relative heart weight), varies considerably (39, 75, 186), by as much as 20-fold (185). The relative heart weight is highest in birds, followed by mammals and by poikilotherms; fish appear to have the lowest heart weight among all vertebrates. The maximum acceleration of heart growth during phylogeny occurs when the metabolic activity of animal tissues has substantially increased, i.e., during transition from poikilothermy to homeothermy (53, 186) (Fig. 2).

It is obvious that phylogenetic differences in cardiac size, performance, and energy demand are reflected in the construction of an oxygen pathway from the blood to mitochondria. The first coronary vessels appeared in some fish at least 500 million years ago (181, 182). Their development is closely related to the transformation of the musculature from a spongy avascular myocardium to a compact myocardium supplied from coronary arteries. Although the heart of adult homeotherms consists entirely of a compact musculature with coronary blood supply, the ventricular myocardium of most species of cold-blooded vertebrates is formed by two different muscular layers: the inner avascular spongious musculature, supplied by diffusion from the ventricular lumen, is covered by an outer compact layer with a coronary blood supply (18, 156, 161–163, 186) (Fig. 3). Quantitative analysis of the terminal blood bed has revealed that poikilotherms with a low relative heart weight (e.g., fish) have a significantly lower lacunar capacity than animals with a higher relative heart weight, such as amphibians and reptiles (160). Recently, Kohmoto et al. (99) have demonstrated a high degree of direct myocardial perfusion from the ventricular cavity in the endocardial region of alligator hearts. The high density of channels and thin sheets of myocardium permit reasonable diffusion distances between myocytes and the ventricular cavity.

The myocardial blood supply of certain lower vertebrates appears to be “primitive” and “inefficient.” However, there may well be good reasons for the principles
concerned to inform clinicians who treat heart disease (28). Medical researchers seeking treatment for blocked coronary arteries are now becoming interested in the spongy myocardium of fishes and reptiles. The information obtained represents a significant step toward achieving transmyocardial blood flow in patients. Surgeons have already begun using lasers to punch holes in the left ventricle to permit some oxygenation directly from the luminal blood, a technique known as transmyocardial revascularization (82). This operative technique may improve myocardial perfusion in patients in whom the standard method of revascularization is counterindicated.

2. Ontogenetic aspects

It is obvious that the type of blood supply changes significantly during phylogenetic development and that coronary arteries play a dominant role only in adult homeotherms, birds, and mammals. In this connection, an obvious question arises about the time course of development of myocardial blood supply during the ontogeny of warm-blooded animals.

The development of the vascular bed in the heart of homeotherms has been summarized by Rakusan (191), Hudlicka et al. (83), and Tomanek (229). Recent studies have shown that capillaries form via vasculogenesis, i.e., from precursor cells, which migrate to the epicardium from the liver region and then form tubes (132, 179). These structures first appear 13 days after conception in rats (206) and during the fourth week of pregnancy in humans (148). The vascular tubes mature and become associated with pericytes; these vessels subsequently grow by angiogenesis, a process which consists of disrup-

![Chart](chart.png)

**FIG. 2.** Relative heart weight in individual classes of vertebrates. [Data from Hesse (75).]

![Diagram](diagram.png)

**FIG. 3.** Different types of myocardial blood supply. *a:* Spongyous musculature supplied from ventricular lumen. *b:* Inner spongyous layer is covered by an outer compact musculature with vascular supply. *c:* As in *b,* but capillaries are present also in some trabecels of spongylie musculature. *d:* Compact musculature supplied from coronary vessels. [Adapted from Ostadal et al. (160).]
tion of the basement membrane and migration and proliferation of endothelial cells to form new channels (229). After early capillarization, plexes of vessels with venular dimensions appear; in contrast, the formation of the arterial system is a later event (77, 209). The major coronary arteries form by coalescence of microvessels that grow toward and penetrate the aorta (20). The coronary arteries connect to the aorta in humans as early as after 44 days of pregnancy (77). The development of the arterial vasculature continues after birth; there is an increase in the number of branches and an increase in the length of arterial segments (44, 198). Similarly, the early postnatal period is characterized by a rapid rate of coronary capillary formation; close to one-half of all adult capillaries in the rat heart are formed during the first 3–4 postnatal weeks, and after 45 days of age in rats, capillary growth stops (149, 194). The ultrastructural differentiation of capillary endothelium in rats is completed by the end of the second postnatal week (163), but the development of various enzymes characteristic of capillary endothelium (ATPase, alkaline phosphatase, and dipeptidyl peptidase IV) is only completed after weaning (116). It is well established that the process of vascularization is influenced by growth factors, the extracellular matrix, and mechanical forces; however, there are few experimental data concerning this issue (229).

The main quantitative changes of the coronary capillary bed during the subsequent period are a decrease in the muscle fiber-to-capillary ratio and a decrease in capillary density; the number of myocytes supplied by a single capillary decreases from four to six in the neonatal period to one in adult hearts from humans, rabbits, and rats; simultaneously, the number of muscle fibers per square millimeter decreases, whereas their diameter increases (182, 193). A decrease in capillary density with increasing age and cardiac mass occurs in all mammalian hearts. It follows that the capillary domain, i.e., the area supplied by a single capillary in a tissue cross section, increases with increasing cardiac mass and is similar in both rats and humans (192).

It may be concluded that changes in the heart size during ontogenetic development of homeotherms are, as during phylogeny, accompanied by the gradual transformation of the avascular spongy musculature into a compact myocardium supplied through coronary vessels. Ontogenetic development of the myocardial blood supply can be thus divided into three periods (164): 1) lacunar, up to the development of coronary arteries the myocardium is entirely spongy and supplied from ventricular cavity; 2) transient, from the beginning of the arterial stem development to the time when the definite coronary vasculature is formed during which the myocardium may be supplied both from the lumen and from the developing coronary bed; and 3) coronary, when the development of coronary vessels is completed the blood supply is effected mostly from coronary arteries.

B. Development of Myocardial Energy Supply

1. Comparative aspects

For the understanding of developmental changes in cardiac sensitivity to oxygen deprivation, a basic knowledge of the development of cardiac metabolism is of crucial importance. Decisive differences in cardiac energy metabolism in adult vertebrates are the result of changes in metabolic activities of animal tissues, the most obvious one taking place during transition from poikilothermy to homeothermy (for review, see Ref. 49). Thermoregulating mammals and birds have a relative heart weight and total metabolic capacity that is, on average, four times whereas arterial blood pressure is six times higher than in poikilotherms (97, 181). In terms of anaerobic metabolism, all requisite enzymes of glycolysis are routinely detected in vigorous activities. Hexokinase, which catalyzes the first step in the utilization of exogenously supplied glucose, is about five times more active in hearts of poikilotherms than homeotherms (49). The mitochondrial enzymes of the poikilotherm and homeotherm heart are similar in their structure and functional properties, but their activity per milligram tissue or tissue protein is considerably lower in cold-blooded hearts (53, 84, 247); for example, reptiles possess only 20–50% of the mammalian capacity (52, 53). The ratio of creatine kinase to cytochrome-c oxidase, a rough estimate of aerobic capacity and cellular energy turnover, is significantly increased in poikilotherms (38). When challenged by hypoxia, this high level may represent an enhanced efficiency to attenuate the impact of a depressed energy liberation. Moreover, poikilothermic hearts possess a high relative glycolytic capacity as indicated by a high pyruvate kinase-to-cytochrome-c oxidase ratio.

Significant metabolic differences exist between the compact and the spongy layer of the poikilothermic heart (15). The activities of enzymes that are connected with aerobic oxidation (citrate synthase, malate dehydrogenase) and glucose phosphorylation (hexokinase) are higher in the spongy than in the compact layer. Similarly, the content of phospholipids is higher in the spongy musculature, the greatest difference being in the content of diphosphatidylglycerol (50). Furthermore, Maresca et al. (121) and Greco et al. (63) have demonstrated that differences in enzyme activities are accompanied by different mitochondrial populations in the two layers. It is interesting to note that the myosin ATPase activity (calcium activated) is significantly higher in the compact musculature as compared with the spongy layer of the carp heart (15).

In conclusion, significant metabolic differences exist
between the poikilothermic and homeothermic heart and even between the two layers of the same heart of lower vertebrates. It is likely that these differences play a crucial role in the different sensitivity of the poikilo- and homeothermic heart to oxygen deficiency.

2. Ontogenetic aspects

Whereas the heart of adult homeotherms can utilize a wide spectrum of substrates for the provision of energy, including lipids, carbohydrates, and amino acids with long-chain fatty acids as the predominant source, fetal metabolism is primarily anaerobic, and this adaptive property is retained during the neonatal period. The high stores of glycogen that characterize the fetal and newborn myocardium are essential for enhancing tolerance to hypoxia, but these decrease rapidly after birth; anaerobic production of energy may also be related to enhanced stores of amino acids that allow substrate level phosphorylation (92). The immature heart mainly depends on glycolysis because the capacity to use fatty acids is impaired due to either delayed maturation of enzymes associated with mitochondrial fatty acid transport and metabolism or to deficiency of carnitine (30, 31, 235–237). In addition to the enzymes associated with fatty acid metabolism, several other enzymes, such as those of the citric acid cycle and the respiratory chain, together with creatine kinase and various cytochromes, have been shown to have a low activity in the immature heart (48, 80, 232, 237). Postnatal development of enzyme activities depends on the degree of maturation of individual species at birth. In rats, highly immature at birth, enzyme activities exhibit differences in the guinea pig, which is more mature at birth, were significantly less pronounced (A. Bass, B. Ostadal, M. Stejskalova, and A. Stiejlerova, unpublished observations; and Ref. 80). Significant differences in enzyme activities were found between the atrial and ventricular myocardium (17, 231); the activity of enzymes, connected with aerobic metabolism, lactate, and fatty acid metabolism, was significantly higher in the ventricular myocardium, whereas that of hexokinase was higher in the atrial tissue. This suggests that the ventricle is amply equipped for utilization and oxidation of all major nutrients (lactate, fatty acids, and glucose), whereas the atrium predominantly utilizes glucose.

Ontogenetic development is characterized by quantitative and qualitative changes in the cardiac mitochondria. There are twofold increases in volume density, size, and number of mitochondria per cell between days 0 and 5 in rats (149) and months 0 and 2 in dogs (109). Moreover, developmental changes in mitochondrial oxygen utilization have been demonstrated (213, 222, 244, 253); as a result, the respiratory control ratio (a measure of the dependence of respiratory rate on ADP) is greater in the mitochondria of fetal and neonate hearts, suggesting that the higher respiratory rates in immature hearts are a reflection of a high level of electron transport.

In conclusion, cardiac metabolism changes in response to oxygen and substrate availability during development. The fetus is relatively more dependent on anaerobic glycolysis, using glucose as its major source, whereas the mature heart is almost exclusively aerobic, with nonesterified fatty acids as the predominant substrate. The fact that the fetal heart relies on carbohydrate metabolism can therefore be regarded as adaptation to hypoxia.

IV. DEVELOPMENT OF CARDIAC SENSITIVITY TO OXYGEN DEPRIVATION

A. Comparative Aspects

Of the experimental models commonly used for studying the effect of oxygen deprivation in homeotherms (see Table 1), only a limited number are suitable for the cold-blooded heart. The absence of, or a partially developed, coronary circulation excludes, for instance, the possibility of using acute or chronic regional ischemia. The most frequently used models are, therefore, systemic and histotoxic hypoxia.

As discussed in section II B, the poikilothermic heart, which is frequently exposed to oxygen deficiency in an aquatic environment, is better equipped biochemically to cope with oxygen deprivation than the mammalian one. Data comparing the sensitivity of the poikilothermic and homeothermic heart to oxygen deficiency are relatively scarce. The anaerobic capacity of cardiac muscle in different chordates from cyclostome (hagfish) to humans has been reviewed by Poupa (181) and Driedzic and Gesser (49). Force development of isometric cardiac strips in vitro under roughly similar conditions was measured when respiration was blocked by cyanide (histotoxic hypoxia). The highest tolerance to lack of oxygen was observed in the reptilian (monitor lizard) and cyclostome
(hagfish) heart; the force development was reduced by no more than 30% and recovered slowly toward initial values. Large differences were found in fish; contractile force in free-swimming cod was reduced by 90%, whereas that in bottom-dwelling flounder was reduced by only 50% (within 45 min after the onset of hypoxia). The highest sensitivity was observed in homeotherms (humans); a decline of contractile force occurred immediately (within 5 min) and was irreversible. In poikilotherms, the sensitivity to histotoxic hypoxia was increased upon increasing the temperature of the perfusion medium (35). Myocardial cells of poikilotherms, similar to as in homeotherms, may be irreversibly damaged by hypoxia (110); highly resistant reptilian heart should be noted as a probable exception (238–240).

Acidosis protects the reptilian heart against contractile dysfunction induced by hypoxia, but no similar effect was observed in the fish heart (60). The ability of the heart to work at high \( \text{PCO}_2 \) is probably another cause of the high resistance of the reptilian myocardium to hypoxia and cannot be explained either by a different tissue pH or by buffering capacity. In view of the fact that calcium competes with protons, it can be speculated that the effect of \( \text{CO}_2 \) is counteracted by calcium activation (181). Because enhanced sarcolemmal calcium influx or increased calcium release from the sarcoplasmic reticulum as a calcium source were excluded in these studies, mitochondrial calcium was proposed as a tentative candidate; by blocking the mitochondrial sodium/calcium exchange, the protective effect of acidosis on reptilian heart was abolished (60). Furthermore, the poikilothermic heart is, in comparison with the homeothermic one, resistant to the calcium paradox. This phenomenon, i.e., the development of mechanical impairment when the heart is reperfused with normal calcium after a brief period of cardiac arrest during perfusion with calcium-free medium (258), has not been observed in ventricular strips from several different species of cold-blooded animals (183).

Because it is well known that calcium plays an important role in the development of ischemia and/or hypoxia-induced myocardial injury, the phylogenetic differences in systems involved in calcium handling have to be taken into consideration as a possible explanation of the difference in the sensitivity of poikilothermic heart to oxygen deprivation. The cardiac coupling mechanism in lower vertebrates relies heavily on sarcolemmal calcium fluxes, whereas the sarcoplasmic reticulum is of little or no importance, e.g., in amphibians (55, 128). In many fish, the sarcoplasmic reticulum certainly conforms to this amphibian pattern, but there are other species in which the sarcoplasmic reticulum is undoubtedly better developed (134, 212). On the other hand, a t-tubular system has never been observed in fish myocardial cells, and thus the concept of functional couplings between cisternae a t tubules in mammals and birds does not apply to myocardial cells in fish (212). The functional significance of myocardial sarcoplasmic reticulum in poikilotherms is unclear and may differ between atrium and ventricle (49).

In conclusion, it appears that the adult poikilothermic heart as a whole (i.e., without separation into the compact and spongious layer) is significantly less sensitive to oxygen deprivation as compared with the homeothermic one, probably because of higher anaerobic capacity and the developmental differences in systems responsible for calcium handling. The important question regarding the relationship between the type of myocardial blood supply (lacunar vs. coronary) and sensitivity to oxygen deprivation was, unfortunately, not addressed in studies done in vitro and remains to be answered.

B. Ontogenetic Aspects

As stated in section IV A, oxygen deprivation in the mammalian myocardium is predominantly induced by ischemia or systemic hypoxia. However, they do not represent equivalent insults. Whereas all flow is terminated in ischemia, hypoxia involves the exposure of the myocardium to a perfusate with low \( \text{PO}_2 \). The latter condition provides a continuous supply of substrate, whereas removal of lactate from the hypoxic heart prevents the intracellular acidosis that occurs with ischemia. Thus, by anaerobic glycolysis, the energy stores and ionic gradients can be maintained in the hypoxic heart for a considerable period of time (187). Over the past years, many experimental studies have compared the tolerance of the mature and immature heart to hypoxia and ischemia (for review, see Ref. 203). These studies were stimulated by the increasing clinical interest in the immature heart of children who have undergone open-heart surgery in which the myocardium is subjected to ischemic arrest and by the early ontogenetic occurrence of the risk factors for ischemic heart disease.

1. Cardiac sensitivity to hypoxia

Early evidence for an age-dependent decrease in resistance to hypoxia is found in studies on the survival time of the rat, cat, dog, guinea pig, and rabbit in anoxic environments (56, 62). It was found that in each species the survival time was inversely related to age and to the maturity of the newborn. The survival time in rabbits decreased day by day even during the first week of life and reached adult values at 2 wk of age (62). The capability of the newborn to withstand hypoxia has been attributed to the high glycogen content in the heart and liver. However, because these studies were performed in the intact animals, the effect of hypoxia on the central nervous system, peripheral resistance, and acid-base balance could indirectly affect myocardial function.

The concept of greater tolerance of the neonatal
heart has been supported by Su and Friedman (225) and Jarmakani and co-workers (86, 89). By using the perfused myocardium, they have shown that 30 min of anoxia (perfusate equilibrated with 95% N₂-5% CO₂) had a minimum effect on myocardial function in the newborn rabbit (0–2 days) and dog (0–9 days); the effect of hypoxia on the contractile function was, in both species, inversely related to age. Moreover, the increase in lactate production during hypoxia was significantly greater in the newborn than in the adult, indicating that the newborns are capable of maintaining adult levels of myocardial ATP. Several other studies have shown a greater posthypoxic preservation of variables, such as calcium handling (88, 141) and mitochondrial function in the newborn heart (254). Similar age-dependent tolerance to hypoxia was observed in rats (157); the resistance of the isolated right ventricle expressed as the recovery of contractility after 20 min nitrogen anoxia (the mixture of 95% O₂-5% CO₂ was replaced with 95% N₂-5% CO₂; Ref. 127) was significantly higher in newborn males than in adults. The ontogenetic changes showed a biphasic pattern. The relatively high cardiac resistance at birth even increased up to the 30th day of life (i.e., up to the end of the weaning period) in both male and female hearts; however, this value decreased in males from the 30th to the 60th day but remained unchanged in females. The adult female heart was thus significantly more resistant to hypoxia (Fig. 4).

It may be concluded that cardiac sensitivity to hypoxia or anoxia changes significantly during ontogenetic development; however, the time course is different in males and females. Detailed information about the development of cardiac tolerance to hypoxia during early postnatal period is unfortunately still lacking.

FIG. 4. Ontogenetic development of cardiac tolerance to acute hypoxia (expressed as recovery of isotonic contraction of isolated right ventricle after 20 min of acute anoxia in vitro) in male and female rats. [Data from Ostadal et al. (157).]

2. Cardiac sensitivity to ischemia

As with hypoxia, the immature myocardium also appears to be relatively resistant to ischemia in the rabbit (9, 27, 64, 146), pig (10), dog (91), and rat (204, 205, 252). Riva and Hearse (205) have observed that the age-dependent changes in resistance to global ischemia in the isolated rat heart (expressed as postischemic recovery of developed pressure) showed a biphasic pattern with increasing tolerance from 5 to 23 days of age, followed by a decline to adulthood. These observations were similar to our experiments on cardiac resistance to acute nitrogen anoxia in vitro (157). Detailed analysis of the tolerance of the isolated rat heart to global ischemia during the first week of life has, however, revealed a significant decrease from day 1 to day 7 (170) (Fig. 5), suggesting a possible triphasic pattern of the ontogenetic development of cardiac sensitivity to ischemia. The sensitivity of neonatal myocardium may be species dependent. Baker et al. (10) have shown that the neonatal pig heart is more susceptible to ischemic injury than the neonatal rabbit heart.

In this connection, it is necessary to mention studies showing that the immature heart might be more susceptible to ischemic injury than the adult heart (37, 187, 250). The above studies reporting similarities between findings under hypoxic and ischemic conditions suggest that the differences between the form of oxygen deprivation used are not sufficient to account for the conflicting results. According to the results of Quantz et al. (189), the reported conclusions were contradictory, primarily because of the different end point used to assess the tolerance. Those who used time to onset of ischemic contracture (TIC) as the end point found it to be shorter in neonatal hearts and thus concluded that the immature hearts are more susceptible to ischemic injury. In contrast, those
who evaluated the recovery of ventricular function when ischemia was followed by a period of reperfusion concluded that the immature heart regained a better function and thus is more tolerant to ischemic insults. The onset of ischemic contracture is thought to mark the beginning of irreversible damage and correlates with the depletion of high-energy phosphates. However, TIC does not reflect events after reperfusion and does not, in fact, indicate the irreversible damage to the global ventricular function. Therefore, TIC is not a good index of ischemic injury. Studying functional recovery after an ischemic insult as the end point seems to be more closely related to situations in cardiac surgery.

3. Possible mechanisms of the higher tolerance of the immature heart

The mechanisms of the higher resistance of the immature heart to oxygen deprivation have not yet been satisfactorily clarified. It may be speculated that an explanation for the phenomenon lies in the lower energy demand, greater anaerobic glycolytic capacity, and higher glycogen reserves of the neonatal heart (79, 254) (Table 2). The ontogenetic difference would also be consistent with biochemical maturation, since many of the striking differences with respect to substrate utilization and energy metabolism are lost soon after birth. The tolerance of the immature heart to ischemia may also be related to amino acid utilization by transamination (92). Moreover, the ATP catabolic pathways change during development (81, 139, 223). During normoxia, the adult heart releases 80% of total purine as urate and 7% as inosine, whereas the immature heart releases 64% as hypoxanthine and 15% as inosine. Hypoxanthine and inosine produced by the immature heart can be recycled into the ATP pool, whereas urate is not recyclable. As a consequence, ATP depletion occurs more rapidly in the mature heart. In addition, AMP accumulates in the immature heart during ischemia, which allows a rapid replenishment of ATP on reperfusion. On the other hand, de novo synthesis of ATP is required in the mature heart. These observations suggest that the immature heart is better adapted to ATP synthesis than to its breakdown, the situation that may be advantageous in conditions of low substrate availability. The immature heart thus suffers less ischemic injury than the mature heart after the same ischemic insult (223).

Another factor that may contribute to the increased tolerance of the immature heart is the age-related change in calcium handling (143, 145, 234, 254). Calcium homeostasis is closely related to cell metabolism and is an accepted determinant of tissue injury during both oxygen deprivation and repletion. Calcium handling in the neonate is different from that in the adult. The contraction of mammalian myocardium is known to depend on both transsarcolemmal calcium influx and calcium release from the sarcoplasmic reticulum (54). However, the relative contribution of the two mechanisms varies significantly during development. The contraction of neonatal myocardium where the sarcoplasmic reticulum is not fully developed depends to a large extent on the influx of calcium across the sarcolemma via the calcium channels. Further development, the ability of sarcoplasmic reticulum to accumulate calcium increases, and there is a progressive maturation of calcium release from the sarcoplasmic reticulum (228). Similarly, calcium sensitivity of cardiac myofilaments increases, reaching the adult values in rats starting from the 15th day of postnatal life (233). An analogous developmental trend has the cardiac sensitivity to isoproterenol-induced calcium overload (169). The role of acidosis may also be important; the negative inotropic effect of low pH was strikingly smaller in the neonatal rabbits (142) which, according to Solaro et al. (221), can be accounted for by a lower myofibrillar calcium sensitivity to low pH in this age group. None of these observations can, however, fully explain the day-by-day changes in cardiac tolerance to ischemia we have observed in rats during the first week of life (170).

Another determinant of tissue injury is oxygen free radicals. According to Southworth et al. (223), the immature heart exposed to an ischemic insult suffers less injury than the mature heart and, as a consequence, produces fewer free radicals on reperfusion. They found that the adult guinea pig heart produced more oxygen radicals than the immature heart (3 days premature delivered by caesarean section) over the range of ischemic durations examined (0–60 min). The largest difference was observed after 20-min ischemia when the mature heart produced four times more free radicals than the immature heart. Because the major source of oxygen radicals in this model involves xanthine oxidase, developmental differences in the capacity for free radical production on reperfusion can be explained in terms of priming for the xanthine oxidase system during ischemia. It is not clear, however, if similar developmental changes occur in the other free radical-generating mechanisms that may be important in the pathogenesis of ischemia-reperfusion injury. Moreover, the exact relationship between free rad-
ical production on reperfusion and the recovery of contractile function remains unclear. It has been shown that the recovery of the contractile function is closely related to ischemic duration rather than to the profile of radical production (223). This suggests that ischemic injury is a more important factor in influencing recovery of cardiac function than is free radical production.

In this connection, it is relevant to explore whether differential susceptibility to reperfusion-induced injury may be partly responsible for the differences in postischemic recovery between neonates and adults (203). Nakanishi et al. (143) compared the consequences of reoxygenation after 40 min of hypoxia (95% N₂) in isolated, arterially perfused, septal preparations of adult and neonatal (3–7 days) rabbits. They have observed that enzyme release during hypoxia and reoxygenation in the newborn was significantly less than in the adult, suggesting less sarcolemmal damage in the young animals. Because the extent of reperfusion-induced injury depends on the severity of the preceding hypoxia or ischemia (59, 71), the likely interpretation of these results is that the neonate sustained less injury during the 40 min of hypoxia than the adult and was thus less vulnerable to reperfusion injury (203). To determine the tolerance to reperfusion-induced injury in the neonatal hearts, studies in which interventions designed to overcome reperfusion-induced injury are given at the time of reperfusion would be desirable (203). To our knowledge, no such studies have been reported in developing hearts.

V. PROTECTION OF THE DEVELOPING HEART

From the foregoing discussion it is evident that the degree of hypoxic injury depends not only on the intensity and duration of the hypoxic stimulus but also on the level of cardiac tolerance to oxygen deprivation. It is, therefore, not surprising that the interest of many experimental and clinical cardiologists during the past 35 years has been focused on the question of how cardiac tolerance to oxygen deprivation might be increased. In this regard, there are three possibilities: 1) pathophysiological interventions that may be long lasting (adaptation to chronic hypoxia) or short lasting (i.e., preconditioning) to counteract the adverse effects of reduced oxygen supply, 2) pharmacological interventions that either increase the myocardial oxygen supply (vasodilators) or reduce oxygen demand (negative inotropes), and 3) cardioplegia, a special type of pharmacological protection, often combined with hypothermia and used in cardiac surgery. Whereas a substantial amount of data are available concerning protection of the adult myocardium, much less is known about this phenomenon in the developing heart. The data in the literature are scarce and often contradictory. The present review summarizes the results and hypotheses dealing with the ontogenetic differences and limitations in endogenous and exogenous protection of the hypoxic/ischemic heart.

A. Adaptation to Chronic Hypoxia

Adaptation to chronic hypoxia is characterized by a variety of functional changes that help to maintain homeostasis with minimum energy expenditure (51). Such adjustment may protect the heart under conditions that require enhanced work and consequently increased metabolism. In addition to such protective effects, adaptation to chronic hypoxia may also exert adverse influences on the cardiopulmonary system, including the development of pulmonary hypertension and right ventricular hypertrophy, which may result in congestive heart failure (for review, see Refs. 26, 100, 137, 154, 165).

1. Protective effect of adaptation

In chronic hypoxia, the myocardium must maintain an adequate contractile function despite the lower oxygen tension in coronary circulation. It was reported in the late 1950s (85) that the incidence of myocardial infarction is lower in people who live at high altitude (Peru, 4,000 m). In addition to chronic hypoxia however, relatively increased physical activity and reduced obesity have to be taken into consideration when explaining the protective effects of living at high altitudes. Epidemiological observations on the protective effect of high altitude are consistent with the results of experimental studies using acute anoxia in vitro, necrogenic doses of isoproterenol, and acute ischemia for testing the myocardial resistance in acclimatized animals. In this connection, it must be pointed out that the pioneer experimental studies on the protective effect of high altitude on cardiac muscle were carried out in Prague by Kopecky and Daum in 1958 (106) and by Poupa et al. in 1966 (184). They demonstrated that the isolated right ventricular myocardium of rats acclimated for at least 6 wk to an intermittent simulated altitude of 7,000 m (24 h every other day) recovers its contractility in vitro after nitrogen anoxia better than the same preparation from normoxic animals. These findings were later confirmed by McGrath and Bullard (126) and Meerson et al. (129). Furthermore, it has been reported (127, 246) that a similar protective effect can be induced by a relatively short intermittent exposure of rats to simulated high altitude (4 h/day, a total of 24 exposures to 7,000 m). Recently, an increased tolerance to global ischemia, manifested as improved recovery of the contractile function following reperfusion, was shown in isolated perfused hearts of adult rats acclimatized to permanent normobaric hypoxia. Furthermore, adaptation to high altitude had a pronounced antiarrhythmic effect under conditions of acute myocardial ischemia (131) and attenuated the
development of systemic hypertension and left ventricular hypertrophy in spontaneously hypertensive rats (73).

A few authors have compared the tolerance of chronically hypoxic versus normoxic immature myocardium. We (155) observed that intermittent high-altitude hypoxia resulted in similarly enhanced cardiac resistance (expressed as the recovery of contractile function of the isolated right ventricle after acute nitrogen anoxia in vitro) in rats exposed to hypoxia either from the fourth day of postnatal life or in adulthood (Fig. 6). Adaptation to chronic hypoxia increased the tolerance in both sexes, but the sex difference (i.e., increased tolerance in females) was maintained (158). An important feature of adaptation to chronic hypoxia is that the protective effect persists even 4 mo after the removal of rats from the hypoxic environment (154). This finding needs further detailed experimental investigation; nevertheless, it suggests further possibilities in the search for effective protection of the myocardium against various types of hypoxic injury. Baker et al. (11) demonstrated that adaptation to chronic hypoxia from birth in a normobaric chamber increased the tolerance of the developing rabbit heart (day 7 to day 28). Recovery of postischemic aortic flow at these stages was better in chronically hypoxic hearts compared with age-matched controls. Furthermore, metabolic adaptation to hypoxemia has been observed in the myocardium of children with congenital cardiac malformations producing hypoxemia (210). The aerobic capacity of the energetic metabolism was significantly reduced in hypoxic hearts as compared with normoxic patients.

It may be, therefore, concluded that adaptation to chronic hypoxia increases cardiac resistance to acute hypoxic injury in both adult and immature hearts. Because most of these effects were observed in acclimatized rats, the behavior of larger immature species under conditions of chronic hypoxia needs to be examined in future investigations.

2. Possible protective mechanisms

A) Blood Oxygen Transport Capacity. An increase in hemoglobin increases the blood oxygen concentration. Indeed, mammals and birds introduced to high altitudes develop variable degrees of polycythemia (199), associated with a shift to oxygen dissociation curve to the right due to increased concentration of 2,3-diphosphoglycerate (135). On the other hand, mammals and birds genotypically adapted to high altitude show a modest or no increase in hematocrit values (12). It can be concluded, therefore, that an increased blood oxygen content may not necessarily be an adaptive parameter. Several studies demonstrated an increased concentration of myoglobin in chronically hypoxic myocardium (for review, see Ref. 137), but significant differences exist depending on species and age.

B) Tissue Oxygen Transport. There is disagreement with respect to the development of myocardial capillaries in animals exposed to hypobaric hypoxia. Whereas Rotta (207), Clark and Smith (40), and Smith and Clark (219) found a decrease in the ventricular capillary density in chronically hypoxic guinea pigs and rats, Miller and Hale (133) found an increased capillary density in both ventricles. Turek et al. (230) described an increase in the hypertrophied right ventricle and no change in the left ventricle. Moravec et al. (136) as well as Banchero et al. (12) found a similar increase only in the left ventricle. Finally, Rakusan et al. (195) and Pietschmann and Bartels (178) found no evidence of increased myocardial capillary density in animals exposed to high altitude. Mathieu-Costello et al. (123–125) showed that correction for sarcomere length is critical to the interpretation of results; capillary length per volume of muscle fiber was not significantly different between lowland and highland deer mouse. Such a variability in results may be due to the selection of experimental animals, their age, as well as duration of chronic hypoxia. Nevertheless, it seems that coronary angiogenesis and increased coronary flow are effective compensatory mechanisms at the beginning of the process of acclimatization; later, their importance may decrease. This view is supported by normal or even decreased coronary blood flow in residents living at high altitude (137).

C) Energetic Metabolism. The protective effect of chronic hypoxia may involve increased capacity of cardiac anaerobic metabolism, increased energy utilization capacity, and possibly selection of metabolic pathways or substrates with a higher energy efficiency that would
decrease the oxygen requirements (137). This view is supported by our findings in chronically hypoxic rats (16) in which both ventricles had a significantly higher capacity for glucose utilization as well as for the synthesis and degradation of lactate. On the other hand, the ability of the heart to break down fatty acids decreased significantly. Chronic hypobaric hypoxia increased the number of cardiac mitochondria and slightly decreased their mean volume (43). Novel-Chate et al. (147) have shown that chronic hypoxia increased cardiac function-to-oxygen consumption ratio and induced phosphocreatine overshoot after \( \beta \)-adrenoceptor stimulation. Furthermore, acclimatization to high altitude lowers the specific activities of several sarcolemmal ATPases and, at the same time, increases their affinity for ATP (257), which permits a more efficient utilization of ATP and helps to prevent membrane transport function under conditions of low energy production. It should be mentioned, however, that it is often impossible to distinguish the direct effect of chronic hypoxia from those of hypertrophy and from other factors, including the nutritional state associated with exposure to high altitude (14).

**D) NEUROHUMORAL REGULATIONS.** Despite increased sympathetic activity (66), chronic hypoxia is associated with impaired response to adrenergic stimulation due to down-regulation of myocardial \( \beta \)-adrenergic receptors (19, 94). One potential mechanism of desensitization to catecholamines in chronic hypoxia appears to involve a decreased functional activity of the \( G \) protein \( \alpha \)-subunit (93). Chronic hypoxia may also lead to increased degradation of catecholamines as indicated by the elevated activity of catechol-O-methyltransferase (118). Chronic hypoxia-induced decreased sensitivity to catecholamines can be seen to be antiarrhythmic and may be responsible for the protective effect of adaptation on isoproterenol-induced necrotic lesions (184). On the other hand, Kacimi et al. (95) have found that chronic hypoxia increases the muscarinic receptor affinity and density, which may contribute to the blunted responsiveness of the heart to catecholamines.

Chronic hypoxia-induced hypothyroidism may be one of the cardioprotective mechanisms, since the hypothyroid myocardium is less susceptible to hypoxia (120). Furthermore, adaptation to high altitude significantly increased the myocardial level of prostaglandins (188). In fact, a single dose of 7-oxo-prostacyclin increased cardiac tolerance to acute nitrogen anoxia in vitro in control and chronically hypoxic rats (256). The protective effect of acclimatization and prostacyclin were, however, additive, suggesting that different mechanisms may be involved. Adaptation to chronic hypoxia has also been shown to induce a decrease in adenosine receptor density without any change in the affinity for the agonist (98), but the possible involvement of adenosine in the protective effect of adaptation to chronic hypoxia is poorly understood. Similarly, the possible role of heat shock proteins (130), remodeling of the ventricles (130), sex hormones, and antioxidant reserve is not fully established. Recently, Kolár et al. (101) have demonstrated that the ATP-dependent potassium channel blocker glibenclamide practically abolished the antiarrhythmic effect of adaptation, suggesting that these channels appear to be involved in the cardioprotection mechanism of chronic hypoxia. The optimum degree of hypoxia and length of acclimatization for cardioprotection are, however, still unclear.

Unfortunately, despite the fact that the first experimental study on the cardioprotective effect of adaptation to chronic hypoxia was published more than 35 years ago, no satisfactory explanation of this important phenomenon has yet been proposed. Furthermore, most of the above hypotheses are based on results from adult animals; the possible age-related differences in the mechanisms involved are still unexplained.

### 3. Adverse effects of adaptation

The literature concerning the influence of age on chronic hypoxia-induced cardiopulmonary changes is neither sufficient nor consistent. Smith et al. (220) reported that young rats exposed to chronic hypobaric hypoxia showed a lower right ventricular hypertrophy than adult animals. Rabinowitch et al. (190) compared cardiopulmonary changes in rats exposed to permanent hypoxia corresponding to the altitude of 5,000 m starting either on the ninth day of life or in adults. These investigators found that the age difference in pulmonary hypertension and right ventricular hypertrophy was not statistically significant. In our experiments (105, 155), an intermittent high-altitude hypoxia (hypobaric chamber, equivalent to 7,000 m, 8 h/day, a total of 24 exposures) induced chronic pulmonary hypertension and right ventricular enlargement in animals exposed either from the fourth day of life or in adulthood. Whereas the pressure elevation in the pulmonary circulation was expressed more in adult hypoxic animals, the right ventricular enlargement was greater in the young group of rats. Right ventricular weight increased linearly with the rise in pulmonary blood pressure in young hypoxic animals \( (r = 0.72) \); this relationship was, however, very loose in adult rats \( (r = 0.16) \). The close correlation between these two parameters in young hypoxic animals may be the consequence of the higher plasticity of the developing heart (e.g., hyperplastic growth of myocytes just after birth). Chronic hypoxia also modulates changes of collagenous proteins. The proportion of this fraction significantly increased, whereas the collagen I-to-III ratio was decreased; this suggested an increased synthesis of collagen III. The elevation of collagen III in young rats was significantly higher (177). It should be mentioned that even severe chronic hypoxia-induced pulmonary hypertension and right ven-
tricular hypertrophy were completely reversible after removal of rats from the hypoxic atmosphere for a sufficiently long period of time (>4 wk; Refs. 16, 74, 103, 165, 201).

B. Ischemic Preconditioning

The most efficient form of temporal protection of the adult myocardium is ischemic preconditioning, first described by Murry et al. (138). This phenomenon was defined as an enhanced tolerance of the heart to a prolonged period of ischemia that is achieved by prior exposure to much shorter periods of the same insult. Preconditioning is manifested by a reduction of myocardial cell injury (138), marked suppression of ventricular arrhythmias (174, 217), and enhanced recovery of contractile function (36) during reperfusion, after a period of prolonged ischemia. Whereas extensive data are available on ischemic preconditioning in the adult myocardium, information on this protective mechanism in the immature heart is very inadequate. We have shown (167, 170) that classical ischemic preconditioning, at least in rats, is not present at birth, and the enhanced recovery of contractile function develops only at the end of the first postnatal week (day 7). The decreasing tolerance of the neonatal heart to ischemia is thus counteracted by the development of an endogenous protective mechanism. Because there were no data on ischemic preconditioning in the immature heart, we have used an experimental protocol (three 3-min periods of global ischemia, each separated by a 5-min period of reperfusion) which in adult rats improved the recovery of contractile function and reduced the infarct size as well as the number of ventricular arrhythmias (113). Nevertheless, it seems that our findings were not influenced by the age-dependent differences in the threshold for protection, since a prolongation of the ischemic episodes from 40 to 60 min did not lead to the development of preconditioning in 1-day-old animals (170). Similarly, Awad et al. (6) have shown that loss of preconditioning in the immature isolated rat heart cannot be overridden by an increased preconditioning stimulus (5-min ischemia + 5-min reperfusion vs. 4× 5-min ischemia and 5-min reperfusion). In addition, they (7) subjected a 4-day-old heart to the pharmacological stimulus of phenylephrine that also failed to protect the immature heart. On the basis of these results, it cannot be excluded, however, that ischemic preconditioning with another index of injury (e.g., infarct size, number of arrhythmias) or in different animal species might have a different developmental time course. Recently, Liu et al. (112) observed that ischemic preconditioning can be induced in 4- to 7-day-old isolated perfused rabbit hearts. Data on the newborns are, however, lacking. Similarly, some "new" methods of preconditioning (108), such as pharmacological protection with adenosine and A1-selective agonists and muscarinic agonist, may have a different ontogenetic development.

Some information on the neonatal hearts has also been obtained in cultured cells; Webster et al. (242) demonstrated that neonatal rat cardiac myocytes preconditioned by a 25-min exposure to hypoxia followed by reoxygenation were protected against membrane damage for up to 6 h of prolonged severe hypoxia, as determined by arachidonic acid release and contractile recovery. In contrast, nonpreconditioned myocytes exhibited a significant hypoxic damage after 2–4 h. It is obvious that contractile failure in tissue culture occurs much more slowly in response to hypoxia than in the working heart, perhaps because of differences in the balance of energy supply and demand between these two situations. Similarly, Ovelgönne et al. (171) have shown that preconditioning can be induced in cultured neonatal myocytes after a 60-min exposure to hypoxia but not by heat shock proteins. The experimental protocol leading to cardioprotection in culture is, however, significantly different from classical preconditioning, and the information thus obtained is difficult to compare with our results.

The precise mechanisms of preconditioning are still unclear but almost certainly involve the initial release of “endogenous myocardial protective substances” (173, 253), which probably include adenosine, bradykinin, prostacyclin, and nitric oxide, as well as the translocation of protein kinase C (PKC) to sarcolemmal and nuclear membranes (255) and, possibly, an effect on ATP-dependent potassium channels (65). The evidence for this hypothesis includes the prevention of preconditioning by inhibition of the activity or translocation of PKC (114, 224) as well as the fact that activators of PKC can mimic the effects of ischemic preconditioning (41, 114). Protein kinase C is a family of closely related serine-threonine protein kinases that can be classified into two major categories. The conventional PKC isoforms (e.g., α, γ) are characterized enzymatically by their requirement for calcium for activation, and the novel PKC isoforms (e.g., δ, ε, ζ) are structurally related to the conventional PKC but do not require calcium for maximum activation. Protein kinase C isoform expression in the rat heart, however, changes significantly during ontogenetic development (208); PKC-α, -δ, and -ζ were detected in 2-day-neonatal hearts, but only a slight immunoreactivity was detected in extracts from the adult hearts. The decline in PKC-α and -δ occurred during the first two postnatal weeks, whereas PKC-ζ had declined even by the second postnatal day. On the other hand, PKC-ε was detected in neonatal as well as in adult hearts. We have observed (170), in accordance with the above results, that PKC isoforms α, δ, ζ, and ε were expressed during the first postnatal week. Moreover, the levels of PKC-α and -δ changed significantly during this period, whereas PKC-α was expressed at a higher level on
day 1 and PKC-δ only on day 7. On the other hand, no sign of translocation of any PKC isoforms under study was observed on day 7, i.e., on the first day of postnatal life when ischemic preconditioning appeared. On the basis of this observation, the role of PKC in the mechanism of preconditioning cannot be excluded; a study of further postnatal development of this protective phenomenon would be decisive in this respect. Furthermore, the simultaneous presence of at least four different isoforms of PKC in the neonatal heart and of only two different isoforms of PKC in the adults might suggest a high degree of age-dependent specificity and flexibility of the signal transduction mechanisms. Webster et al. (242) found that the PKC activator phorbolester mimics the effect of brief episodes of hypoxia in providing protection of neonatal cultured myocytes against prolonged hypoxia.

Repeated brief coronary occlusions depress systolic function and result in prolonged contractile impairment despite the absence of irreversible damage. This posts ischemic myocardial stunning (23), which occurred also in our experiments, was thought to limit the myocardial oxygen demand and thereby preserve cellular viability. There is now, however, sufficient evidence to state conclusively that preconditioning is not a consequence of myocardial stunning (172). Our results (170) support this conclusion, since stunning was present even on postnatal days 1 and 4 when preconditioning was not observed.

Thus it may be concluded that ischemic preconditioning as a potent cardioprotective mechanism is, at least in rats, not a genotypic phenomenon but that it develops very early during postnatal life (Table 3). It is, however, too early to reach a definite conclusion on whether the mechanisms involved in the preconditioning of the immature heart differ or are identical to those of the adult myocardium. It is hoped that further studies of the ontogenetic development of cardiac protection will assist in characterizing the role of key events during ischemic preconditioning of the immature heart.

C. Pharmacological Interventions

There are, in general, two pharmacological possibilities for improving the oxygen homeostasis in the hypoxic or ischemic myocardium; these include increasing the oxygen supply by increasing coronary flow (vasodilators) and reducing the oxygen consumption by decreasing the cardiac contractile function (negative inotropic drugs). The clinical use of this second group in pediatric cardiology is, however, markedly limited because significant ontogenetic differences exist in cardiovascular sensitivity to inotropic agents (for review, see Refs. 4, 47, 152). In this review, we therefore focus on negative inotropic drugs that affect transsarcolemmal calcium fluxes in cardiac muscle.

The contraction of the adult mammalian myocardium is known to be dependent on both transsarcolemmal calcium influx and calcium release from the sarcoplasmic reticulum (55). However, as has been shown in section 1111, the relative contribution of the two mechanisms varies significantly during development. It was proposed that if the immature heart is relatively more dependent on transsarcolemmal calcium entry, then the developing heart may be more sensitive to the negative inotropic effect of calcium channel antagonists (25, 104, 168, 218, 245).

Gibson et al. (61) were the first to report that verapamil depressed cardiac output in intact puppies to a greater extent than that observed in adult dogs. Boucek et al. (25) and Artman et al. (5) demonstrated that the immature rabbit heart has a greater sensitivity not only to the negative inotropic effects of verapamil but also to the effects of nifedipine and diltiazem. Their conclusions were based on a comparison of neonatal and adult hearts, where the observed difference was prominent. Skovranek et al. (218) found that the mortality of verapamil-treated rats was age dependent. The negative inotropic response of the rat isolated right ventricular myocardium was significantly greater in 3-day-old animals than in all older groups (15-, 30-, and 90-day-old rats) (Fig. 7). Similar age-related changes were observed in experiments on isolated perfused rat heart (104); this ontogenetic difference was found under the conditions of both constant perfusion pressure and constant flow and is thus independent of the state of coronary circulation. It seems that ontogenetic differences in the effect of calcium antagonists are not the result of changes in the density and the affinity of nifedipine-binding sites because they attain the adult values in the rat myocardium already during the first week of life. Similarly, the binding characteristics in the rabbit heart did not differ in neonatal and adult age (24, 200). On the other hand, significant ontogenetic differences have been described in the subcellular localization and surface density of dihydropyridine and ryanodine receptors (245).

Thus it may be concluded that the higher sensitivity of the isolated developing heart to calcium channel blockade is a consequence of a higher functional dependence of the immature myocardium on transsarcolemmal calcium

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influx. Further information would be, however, necessary for an understanding of the developmental changes in cardiovascular sensitivity to calcium antagonists in intact animals. The answer may be, namely, the result of combined reactions of developing myocardial cells (inotropy), developing nodal cells (chronotropy), and developing vascular smooth muscle cells (vasodilation). We presume that despite significant interspecies differences, ontogenetic changes in cardiac sensitivity to calcium antagonists may exist in all mammals including humans, depending, however, on the time course of maturation of the systems involved in myocardial and vascular calcium handling. Thus we should like to point to the possible negative consequences of the clinical use of calcium antagonists in the youngest, very sensitive, age group. The clinical importance of this problem results from the repeatedly observed complications of verapamil treatment during the early postnatal period in children: hypotension and shock (1, 180), atrioventricular block and bradycardia (215), and cardiac arrest in hypocalcemic infants (63). All these reported complications were observed in patients up to 1 yr of life.

D. Cardioplegia

Cardioplegia has revolutionized open-heart surgery. This technique, which was developed on the basis of a theoretical approach to the manipulation of ischemic injury, has markedly increased the tolerance to ischemia which in turn has improved operating conditions. Associated with this has been an improvement in postischemic recovery and a reduction of mortality (67, 98, 252). However, this success has been largely in the field of adult cardiac surgery, whereas protection of the immature myocardium during cardiac surgery remains a significant clinical problem. Bull et al. (32) found no difference in the degree of myocardial protection seen in pediatric patients by St. Thomas’ Hospital cardioplegic solution compared with that provided by reperfusion between intermittent periods of aortic cross-clamping. The explanation of inadequate myocardial protection in young hearts may be related in part to cyanosis increasing the vulnerability of the heart to ischemic injury and to the developmental differences in metabolic profiles of adult and immature hearts. Del Nido et al. (45) and Lofland et al. (115) have suggested that the greater drop in ATP content in the young hearts during reperfusion indicates reperfusion injury as a major mechanism of myocardial damage in the young hearts. This rather complicated and controversial topic was reviewed in detail by Riva and Hearse (203).

The results of experimental studies on cardioplegic protection of the immature rabbit heart have revealed that single-dose cardioplegia plus hypothermia is more or equally effective as hypothermia alone (e.g., Ref. 27), whereas multidose cardioplegia plus hypothermia seems to be detrimental (117). According to Magovern et al. (117), the cardioplegic solution used (St. Thomas’ Hospital) has adverse effects in immature hearts at 10°C that are not seen at 28°C. Similarly, Baker et al. (9) demonstrated at 14°C that the St. Thomas’ Hospital solution improved postischemic recovery of function in the mature rabbit heart but decreased it in the immature heart. Analogous results were obtained in immature pigs (42) and guinea pigs (241) which showed that hypothermic potassium cardioplegia inadequately protects the ischemic myocardium. Species-related changes in the deleterious effect of cold multidose crystalloid cardioplegia have been claimed to occur (10); St. Thomas’ Hospital solution protected neonatal pig myocardium, but it appeared to damage the neonatal rabbit heart. For an explanation of these controversial results, the different time course of cardiac maturation has to be taken into consideration. The composition of cardioplegic solutions may play an important role; their formulation was derived from adult animals and based on the normal ionic composition of the extracellular fluid, with sodium and calcium concentrations, the main determinants of cellular calcium exchange, optimally present at moderately reduced levels (9). Because calcium handling and voltage-dependent calcium channel activity differ in neonates (87, 96, 140, 168), the calcium component of the cardioplegic solution may be responsible for different susceptibility and degree of protection. Furthermore, hypertrophy and cyanosis, which are often present in young hearts undergoing cardiac surgery, may alter not only the sensitivity of the heart to ischemia, but also its responsiveness to cardioplegia. In view of the relatively high mortality and

FIG. 7. Negative inotropic effect of calcium antagonist verapamil on isolated right ventricle in rats of different age. [Data from Skovranek et al. (218).]
morbidity associated with pediatric surgery, it is undoubtedly important to clarify these possibilities and to learn more about the mechanisms of developmental changes underlying different sensitivity of immature and adult myocardium to cardioplegia.

As mentioned earlier, cardioplegia is mostly combined with hypothermia. Cooling slows heart rate and metabolism which, in turn, results in a decrease in oxygen consumption (68, 251). Systemic hypothermia with or without circulatory arrest continues to be used for cardiac protection during pediatric heart surgery; it has been reported that in the neonatal heart, hypothermia permits a “safe” ischemic period for at least 90 min (107). Despite this, clinical studies have reported a 10% mortality using deep (15°C) hypothermia, with 50% of these deaths ascribed to acute cardiac insufficiency (111). It would appear, therefore, that not all hearts are afforded equal protection by hypothermia. Wittnich (248) undertook a study of the metabolic effects of various clinically employed levels of hypothermia (25, 19, and 12°C) on 3-day-old piglet heart. As expected, each level of hypothermia extended the time before the onset of ischemic injury in these neonatal hearts. Even with the deepest hypothermia, however, ATP depletion by as much as 25% was seen in the first 30 min of ischemia (249). Although this was significantly less than during 30 min of normothermic ischemia (50%), some ATP use was still present. In a group of hearts that had 50% less ATP than normal, the safe ischemic time afforded by the use of deep hypothermia was significantly reduced. This suggests that hypothermia does not offer equal protection in all immature hearts, especially those with lower ATP levels, including neonatal hearts exposed to severe hypoxia.

VI. CONCLUSIONS AND PERSPECTIVES

The importance of the developmental approach for experimental and clinical cardiology is indisputable. It offers new possibilities in the experimental studies of pathogeny, prevention, and therapy of serious cardiovascular diseases; hypoxic states of the cardiovascular system may serve as a typical example.

What can we gain from studying the heart of lower vertebrates for analysis of pathogenetic mechanisms involved in the hypoxic/ischemic tolerance of the immature mammalian heart? Their cardiovascular system, which in many superficial ways resembles anatomically different ontogenetic periods of homeotherms, shows an amazing morphological functional and metabolic diversity. Surprisingly complex and unexpected patterns of oxygen-supplying systems have been revealed during the last 30 years that cannot be regarded as primitive or inefficient; some of them are, however, still poorly described. The poikilothermic heart represents a unique model for comparison of the tolerance to oxygen deprivation in two precisely defined, developmentally stable layers of the same heart, differing in their structure (compact, spongy), type of blood supply (coronary, lacunar), as well as the capacity of energetic metabolism. Moreover, this complex system is very susceptible to changes in cardiac growth, work load, and metabolic demand. Furthermore, comparative studies have clearly shown that the adult poikilothermic heart is significantly less sensitive to hypoxia as compared with the homeothermic one. The possible mechanisms involve higher anaerobic capacity and developmental differences in system responsible for calcium handling, i.e., a situation resembling the immature mammalian heart. Although it seems that the type of blood supply (coronary, lacunar) does not play a decisive role in the sensitivity to myocardial injury, precise information on the lacunar myocardial blood supply might be surprisingly useful for the development of new techniques for the treatment of human ischemic heart disease. Unfortunately, the data on cardiac structure, function, and metabolism in lower vertebrates have only infrequently been exploited for insights by investigators studying the mechanisms of cardiac sensitivity to oxygen deprivation in developing hearts of homeotherms. We, however, do hope that developmental cardiologists might come to a new appreciation of the amazing physiological potential of supposedly “simple” cardiovascular systems of lower vertebrates.

Ontogenetic research in the field of cardiac hypoxia and ischemia can generally be divided into four closely related areas: 1) study of normal structural, functional, and biochemical development of the cardiovascular system; 2) study of the tolerance of the developing heart to oxygen deprivation; 3) possibilities of protective interventions; and 4) late effects of early hypoxic damage of the cardiovascular system. The great majority of experimental data indicate that the immature mammalian heart is more resistant to oxygen deficiency as compared with adults. There are, however, many controversies concerning the mechanisms of developmental variation in cardiac responsiveness; the obvious discrepancies may be partly due to the different experimental procedures used for studying different animal species with a different time course of maturation. Precise knowledge of individual developmental periods that are critical for cardiac ontogeny is thus crucial for the prediction and explanation of cardiac reactions to oxygen deficiency. Furthermore, experimental data on ontogenetic differences in cardiac responsiveness to hypoxia/ischemia were obtained almost exclusively from healthy individuals. However, such an approach is far from the clinical situations, where interventions applied in the critical developmental periods may have serious consequences for further maturation. The complications observed after the clinical use of
negative inotropic drugs during early cardiac development may serve as warning examples. Developmental cardiology is now in a molecular era, and our understanding of cardiogenesis expands exponentially. Recent advances of new methodology, particularly molecular biology and genetics, may substantially help in the laborious search for a better understanding of the pathogenetic mechanisms that determine the degree of cardiac tolerance to oxygen deprivation as well as mechanisms responsible for different types of cardiac protection. Nevertheless, molecular analysis in developmental cardiology is unthinkable without a comprehensive and well-integrated view of the field.

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