This is to certify that the

thesis entitled

"Myocardial Development and Coronary Vasodilator Reserve in the Guinea Pig Heart"

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physiology

Major professor

Date Nov 25, 1985

O-7639

MYOCARDIAL DEVELOPMENT AND CORONARY VASODILATOR RESERVE IN THE GUINEA PIG HEART

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physiology

1985

ABSTRACT

MYOCARDIAL DEVELOPMENT AND CORONARY VASODILATOR RESERVE IN THE GUINEA PIG HEART

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Basim Shaba Toma

Morphological studies have demonstrated an age-related decrease in capillary density and capillary surface area in the developing heart. However, the consequences of these changes on myocardial perfusion are not known. I tested the hypothesis that the decreased capillary density is associated with a reduction in coronary blood flow reserve. To test this hypothesis, I studied, in the first series of experiments, coronary 1) responses to adenosine and sodium nitroprusside administration, 2) reactive hyperemia, and 3) autoregulatory capacity. We used a Langendorff-perfused heart preparation from guinea pigs of five different age groups (1 week, 1, 2, 12 and 18 months). Data are expressed as mean $\pm$ SEM. Maximal coronary flows ($ml/min.g^{-1}$) in response to adenosine (10^{-6} to 10^{-5} M) infusion are: 27+1.3, 18.5$\pm$1.4, 12.2$\pm$0.4, 10.3$\pm$0.3 and 10.6$\pm$0.8 at 1 weeks, 1, 2, 12 and 18 mnths, respectively, with the flows at 1 week and 1 month significantly higher than those at 2, 12 and 18 months. There is a similar trend for a decreased maximal coronary perfusion in response to sodium nitroprusside (10^{-6} to 10^{-5} M) and following a 45 second occlusion of the coronary inlet flow. Despite the decreased maximal pharmacologic and reactive hyperemic flow reserve, autoregulation of flow is not altered with growth. The pressure-flow relationship exhibits autoregulation between

25 and 55 mmHg perfusion pressure for all but the 1 week age group which autoregulates within a narrower range of pressures (20-45 mmHg). Total maximal coronary flow (ml/min) increases during development; this indicates that the growth of vessels continues with development. However, since coronary perfusion, corrected per unit cardiac mass, significantly decreases, we conclude that the vascular growth lags behind that of the parenchyma.

In the second series of experiments, I examined the influence of age on coronary metabolic vasodilation, myocardial O_2 consumption ($\dot{M}VO_2$) and adenosine release (RADO) during norepinephrine (NE) infusion. Hearts from 1 month (young) and 18 month (adult) guinea pigs (n=6) were used. NE was infused (0.1 ml/min) over a range of $1x10^{-8}$ to $5x10^{-6}$ M perfusate concentration. Data are expressed as mean $\pm$ SEM. Maximum coronary flow [CF (ml/min/g)] was significantly ($p<0.05$) higher for 1 vs 18 month-old hearts (11.9$\pm$0.4 vs 7.6$\pm$0.4). Oxygen delivery [$\dot{D}O_2$ (ulO_2/g/min)] was 210.0$\pm$7.4 vs 141.0$\pm$7.4 for 1 vs 18 month-old hearts, respectively. Maximum values for $\dot{M}VO_2$ (ulO_2/g/min) are 185.0$\pm$5.0 vs 135.0$\pm$9.0 in 1 and 18 month-old hearts, respectively. The maximum peak RADO (pmol/g/min) was: 9086.0$\pm$1969.0 vs 7414.0$\pm$796.0 and maximum steady-state RADO was 2158.0$\pm$583.0 vs 2496.0$\pm$176.0 for young vs adult hearts, respectively. At a given $\dot{M}VO_2$, RADO is significantly higher for the 18 month-old heart, when compared to the 1 month-old group. However, when RADO is plotted against venous PO_2, RADO is higher in the young than in the adult hearts. Venous oxygen tension of the 18 month-old heart was significantly lower than for 1 month-old group during NE infusion (49.0$\pm$7.0 vs 90.0$\pm$7.0 mmHg). In the 18 month-old heart, CF is similar at a given MVO_2 when MVO_2 is raised either by isoproterenol (ISO) or NE.

Data from this study show that 1) at a given $\dot{M}VO_2$, CF and $\dot{D}O_2$ are significantly lower for the adult hearts when compared to the young hearts, 2) RADO and O_2 extraction are significantly augmented for 18 vs 1 month-old hearts, at the same $\dot{M}VO_2$, and 3) the increased RADO in the adult hearts is not related to their lower tissue PO_2. We conclude that 1) metabolic coronary flow reserve is decreased with age during NE stimulation, 2) this is not the result of an enhanced alpha-adrenergic activity with age, and 3) the adult heart may compensate for the decline in CF and $\dot{D}O_2$ by increasing O_2 extraction and increasing RADO to achieve further vasodilation.

DEDICATION

To the memory of my mother, to my family
back home in Iraq, and to my lovely wife, Alham,
and my daughters, Rana and Lora.

ACKNOWLEDGMENTS

I wish to sincerely thank my advisor, Dr. Harvey Sparks, for the guidance and continuous support and encouragement during my graduate education in this institution. I would also like to thank my committee members: Dr. John Chimoskey, Dr. Gregory Fink, Dr. Joseph Meites and Dr. William Spielman for their constructive suggestions.

Special thanks should go to Dr. Roger Wangler, who implanted the idea of studying "aging" in my mind. I also want to thank our "supertechnician", Mr. Gregory D. Romig, for his technical assistance. Thank you to the rest of the laboratory members: Mark Gorman, Miao-Xiang He, Lana Kaiser, Sharon Kelley, Jacob Mufunda, Carol Veltman-Hall and Bridget Whelton for their "moral" support. Thanks to Ms. Linda Friedsberg for typing my dissertation. Thanks also to my friend, Shalal Alnoor, for his help in editing.

I would also like to thank my Government, The University of Baghdad and the College of Veterinary Medicine for providing me this opportunity to study in this institution.

Finally, my deep thanks should go to my lovely wife, Alham, and to my daughters, Rana and Lora, for their endless love, support, encouragement and patience.

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CHAPTER I INTRODUCTION

The blood supply to the heart must necessarily be an efficient and rapidly adaptable one, in order to meet the various long-continued work loads or sudden increased demands for performance that are thrown upon it during different stages of growth and adult life. In particular, an adequate vascularity relative to the muscle fibers being supplied is of considerable importance.

A number of important changes in the fiber-capillary relationship take place during physiological growth of the mammalian heart. Several morphological studies on different species of experimental animals and on human beings have revealed a gradual decline in fiber-to-capillary ratio of the developing heart, as a result of capillary proliferation (Shiply et al., 1937; Wearn, 1940; Roberts and Wearn, 1941; Rakusan et al., 1965; Tomanek, 1970; Olivetti et al., 1980; Tomanek and Hovanec, 1981). However, the maturational process is also associated with muscle fiber hypertrophy, which results in a decreased capillary density and capillary surface area per mass of the growing heart (Shiply et al., 1937; Roberts and Wearn, 1941; Wearn, 1941; Rakusan et al., 1967; Tomanek, 1970; Henguell et al., 1976; Lund and Tomanek, 1980; Olivetti et al., 1980; Tomanek and Hovanec, 1981; Korecky et al., 1982; Tomanek et al., 1982).

It is also well established that the developmental process is associated with a decline in: myocardial performance (Weisfeldt, 1975; Lakatta, 1979; Kennedy and Caird, 1981; Lakatta and Yin, 1982), response to various autonomic agents in the heart (Lakatta and Yin, 1982, Mace and Levy, 1983) and blood vessels (Altura and Altura, 1977; Fliesch,

1977 and 1980). The developmental process is also associated with a reduced capacity for energy production which is related to adenine nucleotide metabolism in the myocardium (Nohl, 1980; Victoria et al, 1981; Awad and Clay, 1982; DeTata et al., 1985).

These and other age-related changes in myocardial structure and function will be considered in further details in the next section.

The physiologic consequences of age-related changes in myocardial vascularization, responses to autonomic agents and adenine nucleotide metabolism, on the myocardial perfusion have not been studied. This study was designed to investigate the influences of the age-related structural and functional changes on the coronary vasodilator reserve of the developing guinea pig heart. This thesis includes two main studies (reported in chapters 3 and 4); in which the coronary vasodilator reserve was assessed pharmacologically (Chapter 3) and metabolically (Chapter 4). Each study has its own introduction, methods, a result section and a discussion of its specific findings. Chapter 5 is a general discussion of the two studies which includes a recapitulation and summary of the major findings and their interpretation. In addition, it includes a number of ideas and suggestions for future studies along this line.

CHAPTER II REVIEW OF LITERATURE

1. Developmental changes in fiber-capillary relationship:

1.a. Fiber/capillary ratio:

In their early studies on rabbit's hearts. Shiply et al. (1937) found that at the time of birth, the muscle fibers are very small, with a cross-sectional diameter of about 7 u. During growth, the length increases and the cross-sectional diameter becomes greater. This process is a continuous one until adult life is reached, at which time the diameter averages about 21 u, the value sustained throughout the adult life of the rabbit. Such an increase in fiber dimensions results in a sevenfold increase in fibers cross-sectional area. If new capillaries were not formed during this process, the capillary density would be reduced to one-seventh of the original concentration. However, this is not the case. At the time of birth, the fiber-to-capillary ratio (F/C ratio, which is defined as the number of muscle fibers per number of capillaries) is 6:1. As the muscle fiber enlarges, there is a gradual decline in this ratio, as a result of capillary proliferation, until it reaches unity when growth is completed.

Similar findings are reported for humans' hearts by Wearn (1940 and 1941) and Roberts and Wearn (1941). They found that the fiber diameter of hearts of infants averages about 7 u and reaches to 14 u in the adult. The F/C ratio of a 3 week-old infant is 4:1 and declines gradually to 2:1 at 15 years of age and reaches unity when growth is completed at about 20 years of age. An increase in the number of capillaries in guinea pigs' hearts during growth is also reported by

Petren and Sylven in 1937. More recently, a number of studies on rat hearts showed similar changes in fiber-capillary relationship during development. Rakusan et al. (1965) characterized three developmental stages of the rat heart. The first is between birth and 4 weeks when both the number of muscle fibers and capillaries increase and the F/C ratio decreases from 4:1 to 2:1. The second is between 4 and 7 weeks when the number of fibers is constant, but capillaries still rises causing the F/C ratio to approach 1:1. The third is after 7 weeks, when both the number of fibers and capillaries is constant, and the F/C ratio is unity. These findings were confirmed later on by several other studies (Rakusan et al., 1967; Tomanek, 1970; Olivetti et al., 1980). Thus it appears that the process of myocardial fiber growth is associated with an increase in the number of capillaries supplying the mammalian hearts, resulting in a gradual decline in F/C ratio with development.

1.b.: Capillary density:

The decline in F/C ratio from 6:1 to unity indicates that the one capillary, which at birth supplied 4 to 6 small muscle fibers, in the adult supplies only one fiber, the area of which is approximately 4 to 6 times that of birth. In view of the increasing mass of muscle and the changes in the F/C ratio during growth, it is interesting to observe the effect of this change upon the concentration of capillaries in a square millimeter of heart muscle (i.e. the capillary density). In the rabbits' hearts Wearn and his coworkers (Shiply, Shiply and Wearn, 1937; and Wearn, 1940) reported that the capillary density (CD) was not affected by the changes in muscle mass. Their results showed that the

CD ranged between 3000 and 4000 capillary/mm^2, with an average of 3420 $\pm$ 39 capillary/mm^2 from the time of birth until adulthood. However, a more recent study by Rakusan et al. (1967) showed a significant reduction in CD of rabbit hearts. Furthermore, the studies of Wearn and Coworkers (Wearn, 1940 and 1941 and Roberts and Wearn, 1941) on human's hearts, showed a significant decrease in CD (capillary/mm^2) from 4513 and 4458 in 3 week- and 3 month-old child as compared to 3700 and 3400 at 11 and 16 yers-old subjects, respectively. The CD did not change significantly beyond this age.

Several studies on rat heart development have shown a similar reduction in the CD in the developing heart (Rakusan et al., 1965; Tomanek, 1970; Lund and Tomanek, 1978; Olivetti et al, 1980; Tomanek and Hovanec, 1981; Tomanek et al., 1982). The last authors reported that at about 1 month of age, the CD averaged about 5000 capillaries/mm^2 and decreased with growth to about 4000 at 2.5 months of age. These same authors also showed that capillary surface area (um^2) was similarly reduced. Tomanek and Hovanec (1981) demonstrated that the decreased capillary density is more pronounced in the subendocardial than subepicardial region.

Thus it appears that, despite the increase in the total number of capillaries during normal growth, the capillary density and capillary surface area of the developing heart are significantly reduced. This suggests that capillary proliferation lags behind the growth of muscle fibers.

1.c.: Intercapillary distance for diffusion:

It is of interest to examine the effects of decreased CD and CSA in association with the increased muscle fiber mass on diffusion distance of the developing heart. During development, the faster rate of muscle fiber growth, and resultant increase in fiber diameter, with no concomitant increase in capillary supply, will tend to "push" the capillaries farther apart and increase the intercapillary diffusion distance. Several studies on the developing rat hearts have actually confirmed this.

Rakusan et al. (1965) calculated the diffusion distance (as half the distance between two adjoining capillaries) in histological sections of rat hearts. They showed that the diffusion distance develops in two steps. The first stage is relatively long (3 weeks) and varies between 8 and 9 u. The second step starts at 20 days, when the diffusion distance is shortest (mean 8 u), and increases progressively to 9, 10 and 11 microns by 1,2 and 2.5 months of age, respectively. Henquell et al. (1976) filmed in situ right and left ventricles of rats, ranging from 40 to 400 days of age, in order to measure intercapillary distance (ICD). These investigators showed that the ICD (measured as the distance between RBCs) increases from 10.3 um in the 40 day-old group to 12.8 and 17.5 um at 100 and 300 days of age, respectively. Thus between 40 days to 300 days, there is about 70% increase in ICD which is parallel to about 50% decrease in capillary density. This group calculates that the increased ICD results in reduced tissue PO_2 (Henquell et al, 1976). There is also a decreased reserve of capillaries to be recruited especially during increased cardiac performance (Rakusan and Korecky, 1982). The increase in cardiac ICD

with development has also been reported by several other investigators (Olivetti et al., 1980; Rakusan et al., 1967; Rakusan and Korecky, 1982; Tomanek et al., 1982).

In summary, the developmental changes in the fiber-capillary relationship of the mammalian heart results in significant decreases in F/C ratio, CD and capillary surface area (CSA) and increase in ICD for diffusion. Furthermore, these changes exert a limiting effect on the degree of capillary recruitment and tissue oxygenation during increased myocardial performance.

2. Changes in response to autonomic agents of the developing heart:

2.a.: The sympathetic agents:

It is widely recognized that the capacity of the cardiovascular system to adapt to stress declines with advancing age both in human beings and in experimental animals (Lakatta, 1979 and 1980; Lakatta and Yin, 1982). Such changes are manifested by the inability of older subjects to raise the heart rate (HR) and cardiac output (CO), to the same extent as young ones in response to exercise and to beta-adrenergic stimulation.

Conway (1970) used the cardiogreen dilution method to determine the CO in young (18 to 32 years) and old (50 to 65 years) subjects. There were no differences in the resting HR, but the resting cardiac index (CO/m^2 of surface area) was lower in the older group. Administration of propranolol (0.1 to 0.12 ug/kg) resulted in a greater fall in cardiac index in the younger group, but no significant difference in HR between the two groups. The subjects were also exercised on a cyclergometer. The exercise was progressive, with an initial load of 300 kg/min for 4

minutes, which was increased by 150 kg/min at 4 minute intervals until the subject was unable to exercise further. The results showed that CO was lower in the older group at all levels of exercise before beta-blockade. However, during propranolol infusion, the CO curve became almost identical in the young and old groups with increasing levels of exercise. Thus propranolol caused a significantly greater reduction in CO in young than in the older subjects. Conway (1970) interpreted these results to be due to reduced sympathetic stimulus or diminished cardiac response to sympathetic discharge with aging. Vestal et al. (1979) administered several doses of isoproterenol (ISO) intravenously into 27 subjects aged 21 to 73 years. These investigators found that the dose of ISO required to increase the resting HR by 25 beats/min (I_{25}) in the presence or absence of propranolol, increased with age. It was concluded from this study that the beta-adrenoceptor responsiveness to both agonist and antagonist is diminished with age. The age-related decrease in resting and catecholamine-stimulated increase in HR and CO of older subjects, has been reported by several other investigators (Lakatta, 1979 and 1980; Kennedy and Caird, 1981; Kostis et al., 1982; Kuikka and Lansimie, 1982; Fujino et al., 1983; Wilki et al., 1985).

Shock and coworkers (Shriener et al., 1967; Weisfeldt et al., 1968; Shriener et al., 1969) using a heart-lung preparation demonstrated that the cadiac performance was significantly lower for 24 month-old rats as compared to 12 month age group. The left ventricular work index was calculated from the mean blood pressure and aortic outflow. Although hearts of both age groups exhibited decreases in aortic flow and left ventricular index with time, after setting up the preparation, their

decline was faster in the 24 month-old rats. Furthermore, aortic flow was significantly lower for the 24 month-old group at any level of myocardial performance. These investigators also observed that the older group developed ventricular dilatation with little hypertrophy. They concluded that the lower aortic flow and the left ventricular dilation with little relative hypertrophy account for the diminished cardiac performance of the old group. Lee et al. (1972) studied hearts of 6,12 and 24 month-old rats using an open chest preparation. These investigators used the product of aortic flow and mean arterial pressure minus the left ventricular end diastolic pressure as the left ventricular cardiac index (LVCI). During increasing the preload with dextran infusion, the LVCI was insignificantly lower for the 24 month-old hearts. Also, when the afterload was increased with angiotensin II, the LVCI was significantly smaller in 24 month-old rats than 6- and 12-month old rats. These authors concluded that the decreased O_2 availability and ventricular dilatation may partially account for the lower work capacity of the hearts in the older rats during increased afterload. An age-related decline in the inotropic response to catecholamines of isolated muscle trabeculae was shown when 6, 12 and 24 month-old rat heart were studied (Lakatta et al., 1975; Weisfeldt, 1975; Lakatta, 1979 and 1980; Guarnieri et al, 1980; Lakatta and Yin, 1982).

The reported age-related decrease in the chronotropic and inotropic response to catecholamines is confined to ages ranging from adulthood (about 6 months) up to senescence (more than 20 months) rats. There are relatively few studies reported on age-related changes in cardiac performance and responsiveness to catecholamines during early

development. Corre et al. (1976) studied the changes in HR of 5 and 19 week-old rats during maximum exercise on a treadmill. These investigators reported that both resting and maximum HR were significantly lower for the 19 week-old group. Furthermore, Abrass et al (1982) studied the resting and maximum HR in response to ISO in rats of 3, 5, 12 and 24 months of age. These investigators found a similar age-related decrease in both resting and maximum HR during ISO infusion. A decrease in cardiac performance early in life was also reported in rabbits (Frolkis et al., 1975) and dogs (Yin et al, 1976 and Yin et al., 1979).

Thus it appears that there is an age-related decreased in cardiac performance during the early stages of development which continues until senescence. This decrease in cardiac performance is manifested by the decreased responsiveness to stressful situations such as maximum exercise and catecholamine administration.

Attempts have been made by several investigators to elucidate the mechanism(s) underlying the decreased responsiveness of the beta-adrenergic receptors of the heart. The diminished contractile response to catecholamines was demonstrated in isolated cardiac muscle (Lakatta et al., 1975). Furthermore, Corre et al. (1976) reported an age-related decrease in HR in response to several maneuvers including atrial stretching with saline infusion, and administration of propranolol, atropine and atropine plus propranolol. Based on this information, it was concluded that the reduction in the resting and maximum HR is due to intrinsic changes in myocardium as opposed to changes in neural influences.

Williams and Thompson (1973) reported that the adenyl cyclase-cAMP system of several tissues of rat, including the heart, undergo important maturational changes. Cardiac adenyl and guanyl cyclase activities start to decrease at 70 days of age. The activities of cAMP and cGMP phosphodiesterase decrease as well. However, these investigators did not correlate these biochemical changes with functional changes of the heart. Nevertheless, these findings serve as a base for understanding the age-related decrease in the cardiac beta-adrenergic activity. Recently, several groups including Guarnieri et al. (1980), Abrass et al. (1982), Narayanan and Derby (1982) and O'Connor et al. (1983) found that the decreased beta-adrenergic responsiveness is not mediated by decreased density of beta-adrenergic receptors. While Guarnieri et al. (1980), Abrass et al. (1982) and O'Connor et al. (1983) found that the affinity of the receptor does not change with age, Narayanan and Derby (1982) reported that the affinity of beta-receptors does decrease with age. Therefore, there is a general agreement that the beta-receptor component of the system is not altered with age. This led many investigators to study the response of adenyl cyclase-cAMP (AC-cAMP) component of the system. Guarnieri et al. (1980) found that cAMP content and cAMP-dependent protein kinase activity were similar in young and old rat hearts, during both control and in response to ISO. These authors also found that myocardial contractile response was reduced in response to ISO and dibutyryl cAMP, but not to Ca^{++}, with age. Based on these findings, Guarnieri et al. (1980) concluded that the AC-cAMP is not altered with age, and that the factors which limit contractile response to catecholamines in the old hearts act subsequent to protein kinase activation but proximal to the Ca^{++}-troponin interaction.

Furthermore, they also suggested that the release of Ca^{++} in response to excitation is probably enhanced to a greater extent by protein kinase activation in the adult than in the senescent myocardium. In contrast to these findings, Narayanan and Derby (1982) and O'Connor et al. (1983) provided evidence that the decline in myocardial response to catecholamines is the result of the decreased activity of the catalytic subunit of the enzyme AC, since the beta-independent activation of the enzyme by GTP and NaF was similarly reduced in the aged heart. Thus it appears that the decreased capacity of the developing heart to respond to stimuli may be mediated, at least in part, by the decreased responsiveness of the adenyl cyclase-cAMP system to intrinsic catecholamines (as during exercise) or to exogenously administered catecholamines, since the number of beta-receptors did not change with age.

2. b. Parasympathetic agonists:

There have been fewer studies of changes in the parasympathetic cholinergic system during development. Mace and Levy (1983) demonstrated that the heart rate responses to vagal stimulation and acetylcholine (ACh) infusion were much less pronounced in 35 day-old puppies than in adult dogs. The densities of the cholinergic fibers in the SA and AV nodes and in atrial and ventricular myocardium in the newborn were not different from the adult animals. However, the ACh concentration in atria was less in newborn than in adults. On the other hand, Narayanan and Derby (1983) showed that the muscarinic receptors density was significantly greater (24 to 29%) in atria of 24 month-old rats than in atria of 7 to 8 or 3 to 4 month-old rats. Baker et al.

(1985) confirmed these findings in rat ventricular tissue. These investigators found that the total ventricular content of muscarinic receptors was increased by 47% and 37% in the 12 and 24 month-old groups, respectively, as compared with the 3 month-old animals.

ACh and muscarinic agonists attenuate beta-adrenergic stimulation of cardiac activity (Watanbe and Besch, 1975). Based on this and on the findings of the changes in muscarinic receptors mentioned above, it is possible that the age-related decline in adrenergic response of the developing heart, reported earlier, may be mediated, at least in part, by the increased cholinergic activity during aging and development; perhaps by facilitating the antiadrenergic effects of ACh.

2.c. Developmental changes in the reactivity of vascular smooth muscle:

2.c.i.: Beta-adrenergic activity:

The only study on developmental changes in the reactivity of the coronary blood vessels was published by Siro-Brigiani and Chieppa (1965). These investigators showed that isoproterenol (ISO) caused relaxation in the coronary vascular strips of young horses but not those of adult horses.

Beginning five years later, several papers were published on age-related changes in the responsiveness of aortic strips isolated from a variety of experimental animals. Fleisch et al. (1970) reported that the relaxation of aortic strips from young (41 to 60 days) rats to ISO was greater than that of strips from an older group (78 to 90 days old). Aortas from animals 205 to 255 days-old did not respond to ISO. There were no significant differences between the responses of aortas of male and female rats. There was a similar age-related decrease in relaxation

in response to sodium nitrate. However, the difference was significant only at low doses of $NaNO_2$. Rabbit aortas also exhibited an age-related decrease in the relaxing effects of ISO. Aortas from rabbit 56 to 70 days old showed the greatest response, and the vessels isolated from rabbits 690 to 1835 day old were totally nonresponsive. Intermediate responses were obtained from aortas from rabbits of ages in between. Since relaxation in response to $NaNO_2$ was less affected by age than was ISO, Fleisch et al. (1970) concluded that the beta-adrenergic receptor activity decreases with development.

To test whether the decreased response to ISO in older animals is due to decreased cAMP formation, Ericsson (1972 and 1973) measured cAMP levels after exposure to ISO in rat aortic strips of 1,3 and 6 months of age. ISO relaxed the 1 month aortas, but had little relaxing effect on aortas from 3 and 6 month rats. Exogenous cAMP had a similar effect. ISO increased cAMP content of 1 month but not 3 and 6 month old aortas. The author concluded that the reduced effect of ISO was a combined effect of a reduced formation of cAMP and a decreased sensitivity to cAMP. A similar conclusion was drawn from a study by Cohen and Berkowitz (1974) in which they found that the action of exogenous cAMP and cGMP as well as their non-metabolizable analogues was age-dependent. Using the same line of thinking, Ericsson and Lundholm (1975) found that cAMP content, in response to ISO, was significantly lower in aortas from 6 month-old rats in comparison to 1 month old rat aortas. This was not the result of an enhanced hydrolysis of the cyclic nucleotide, because they found the phosphodiesterase (PDE) activity also declines with age. The decreased ability of ISO to raise cAMP content with age was not the result of decreased AC activity either, since NaF-stimulation raised

cAMP content in aortas from old animals to levels significantly higher than those from young animals. In experiments by Cohen et al. (1977), ISO increased cAMP similarly in aortas from 3 to 5 and 9 to 13 week-old rats. However, vessels from 9 to 13 week old rats did not relax as well to ISO as did those from 3 to 5 week-old animals. These results and the results of Ericsson and Lundholm (1975) and the fact that the effect of exogenous cAMP is age-dependent (Cohen et al., 1977) suggest that the diminished aortic relaxation to ISO with age is not due to reduced ability of ISO to increase cAMP, rather it is due to reduced ability of cAMP to induce relaxation.

The decreased effect of ISO with age is not specific to the aorta. Fleisch and Hooker (1976) showed that pulmonary artery strips have a similar age-dependent response to ISO. However, the developmental process did not influence the response of rabbits and rat portal vein to ISO. This suggests that the arteries and veins age in a different manner (Fleisch, 1977).

Altura and Altura (1977) studied mesenteric terminal arterioles from 3 to 4 week, 6 to 8 week, and 12 to 16 month-old rats, and showed a reduction in dilation in response to ISO with increasing age. The changes in vascular beta-adrenergic activity have also been detected during the prenatal life. Fleisch (1980) reported a rise in the arterial response to ISO after parturition followed by a steady decline with increasing age. Beta-adrenergic receptor sensitivity was also shown to be reduced with age in cultured vascular smooth muscles. Volicer et al. (1983) showed that the elevation of cAMP in response to ISO was greater in cells obtained from 24 month-old rat aortas than

those of 36 month-old. In summary, it appears that the beta-adrenergic activity of arterial smooth muscle decreases gradually with maturation from time of birth until adulthood.

2.c.ii: Alpha-adrenergic activity:

Whereas beta receptor mediated responses of the cardiovascular system have been studied in detail, the alpha receptor responses, as well as the response to other vasoactive agents are not extensively documented. Accumulating evidence indicates that the alpha-adrenergic responsiveness of the aortas of several species varies with age (Tuttle, 1966; Cohen and Berkowitz, 1974, 1975, and 1976; Buhler et al., 1980; Fleisch, 1980; Elliot et al., 1982). Tuttle (1966) studied the effect of NE on aortic strips from rats 100 to 135 days, 340 to 375 days and 700 to 810 days old. The maximum force generated was greatest in 1 year old aortas, less in the 100 day old vessels, and lowest in the 2 years old aortas. These results suggest that the alpha-adrenergic response increases initially up to 1 year of age, and gradually declines thereafter. Cohen and Berkowitz (1974 and 1975) showed that NE, apomorphine and dopamine caused higher force of contraction in aortic strips from 9 to 13 week-old rats than those from 3 to 5 week-old strips. Fleisch (1980) reported that adult canine vascular strips were more sensitive to NE, phenylephrine and tyramine than those of the neonate. Buhler et al. (1980) and Elliot et al. (1982) showed evidence of an increased alpha-adrenergic response in subjects of 66 to 78 years in comparison to young subjects (20 to 32 years). However, a recent study by Duckles et al. (1985) showed that neither the accumulation of ^{3}H-NE, nor the contractile response of strips isolated from femoral and

renal arteries and veins of 6, 12, 20 and 27 month-old rats, changes with age. It appears that the results of studies on developmental changes in the alpha-adrenergic responsiveness are still conflicting. However, there is a general agreement that responsiveness increases during the early stages of development.

2.c.iii.: Histamine and serotonin:

Developmental changes in the vascular response to histamine have also been reported. Holl (1977) demonstrated that H_2 receptor-mediated relaxation of rabbit aorta decreases with increasing age, whereas H_1 receptor-mediated contraction did not show significant age-related differences.

Serotonin's action on vascular smooth muscle tends to vary among species and among experiments on animals of the same species. However, for the most part serotonin contracts isolated blood vessels (Fleisch, 1980). The effect of age of vascular responses to this amine is controversial. Hayashi and Toda (1978) demonstrated a marked reduction in the tension development to serotonin in rabbit aorta with age. Aortas from 3 month to 1 year old rabbits showed a progressive decline in sensitivity to serotonin; the ED_{50} increased 6.9 fold. This was not the case in the rat aorta in which the ED_{50} ratio was only 1.2 when aortas from 36 to 52 weeks old vs 6 to 8 week-old rats were compared (Cohen and Berkowitz, 1976).

2.c.iv. Other drugs:

The influence of maturation and development on responses of vascular smooth muscle to ACh has not been extensively studied. Preliminary

studies by Fleisch (1980) using the perfused rat mesenteric vascular bed showed that ACh-induced dilation is not dependent on age. However, a recent study of aortic strips (Hynes and Duckles, 1985) showed that the relaxation of aortic strips from 20 month-old-rats was only about one-third that of 6 month-old rats.

Fleisch (1980) reported that isolated lamb carotid arteries exhibited increased sensitivity to angiotensin II (AII) with age. Tissues from adults were 6 times more sensitive to AII than those from 1 day-old lambs. In the same study, Fleisch (1980) reported that changes in blood pressure induced by prostaglandins (PG) are age-dependent. PGE_2 lowers blood pressure in immature (1 to 2 weeks) as well as mature (1 to 2 months) rats. In contrast PGF_2 alpha lowered blood pressure in young rats but raised pressure in older animals. These results suggest that the PG receptors became increasingly differentiated as the animal develops.

3. Developmental Changes in Adenine Nucleotide Metabolism

3.a.: Role of adenosine in the regulation of coronary blood flow

Before considering the discussion of changes in adenine nucleotide metabolism, the role of an adenine nucleotide metabolite, namely the nucleoside adenosine, in the regulation of coronary blood flow (CBF) will be presented below.

Increased myocardial metabolism is normally accompanied by an increase in CBF and oxygen delivery ($\dot{D}O_2$). The coupling of coronary vascular conductance to metabolism is believed to be mediated by the release of vasodilator substance(s), that lead(s) to reduction in coronary vascular resistance and increase(s) CBF and $\dot{D}O_2$.

The role of adenosine in mediating the increased CBF during increased myocardial metabolism was first proposed by Berne (1963). He hypothesized that the reduction in myocardial oxygen tension during increased myocardial oxygen utilization leads to the breakdown of heart muscle adenine nucleotides to adenosine (ado). Then, ado diffuses out of the cell and reaches the coronary arterioles via the interstitial fluid and produces arteriolar dilation. The resultant increase in CBF elevates myocardial O_2 tension, reducing the rate of degradation of adenine nucleotides, and washes out interstitial ado. Since 1963 the ado hypothesis attracted the interest of many investigators and it has been critically evaluated and tested by several studies. The results of stduies in which ado hypothesis was tested have been periodically reviewed (Berne, 1964; Belloni, 1979; Berne, 1980; Feigl, 1983; Sparks, et al., 1984).

The ado hypothesis has not been fully accepted yet, but there are several findings, in its favor. First, ado is a potent vasodilator (Berne, 1963; Schnaar and Sparks, 1972). Second, the metabolic machinery capable of rapid formation and destruction of ado is present in the myocardium (Wiedmeier et al., 1972); and third, most stimuli which result in increased myocardial metabolism are associated with increased tissue content of ado (McKenzie et al., 1980), pericardial fluid ado concentration (Miller et al., 1979), and increased release of adenosine in the venuos effluent (Wiedmeier and Spell, 1977).

However, there are other lines of evidence against the ado hypothesis. First, Jones et al. (1982) showed that aminophylline, an ado receptor blocker, does not reduce functional hyperemia. Second, Manfredi and Sparks (1982) and Bardenheuer and Schrader (1983)

demonstrated that myocardial oxygen consumption can be raised by electrical pacing or increasing the afterload, without a concomitent increase in adenosine release; and third, DeWitt et al. (1983) showed that the time-course of increased ado release does not match the increase in coronary flow and O_2 consumption during stimulation with catecholamines. In view of these controversial findings, Sparks et al. (1984) suggested that because of the lack of knowledge of the contribution of coronary endothelium to ado metabolism, a final conclusion cannot be drawn at present regarding the role of ado in mediating functional hyperemia. Nevertheless, ado remains the strongest candidate for the cause of steady-state increase in CBF during increased myocardial metabolism.

3.b.: Changes in adenine nucleotide metabolism:

3.b.i: Sources and sinks of adenosine.

The sources and sinks of adenosine production and degradation has been recently reviewed by Sparks and Bardenheuer (1985). These investigators reported that there are at least two sources for ado production, i.e. the adenine nucleotide pool(s) and S-adenosylhomocysteine (SAH).

AMP serves as an immediate percursor of ado. The conversion of AMP to ado is catalyzed by the enzyme 5'-nucleotidase (5' NT). This enzyme is present at least in three forms. First, a membrane bound form (ecto.5'.NT) which is responsible for ado production from extracellular AMP (Frick and Lowenstein, 1976) second, a cytosolic form (endo 5'NT) which catalyzes the conversion of intracellualr AMP to ado (Worku and Newby, 1983) and third, a mitochondrial form (Collinson et al., 1985).

AMP can be found in both the intracellular and extracellular fluid. Intracellular AMP is divided into at least three separate pools. First is cytosolic AMP which is in equilibrium with ADP and ATP. Second is AMP formed from cAMP, catalyzed by phosphodiesterase (Schrader and Gerlach, 1976). The third pool is within mitochondria (Bukoski et al, 1983; Bunger et al, 1983). The extracellular sources of AMP are the adenine nucleotides released from platelets (Fukami et al., 1976), nerves (Burnstock, 1972), cardiac myocytes (Forrester and Williams, 1977) and endothelial cells (Pearson et al, 1980).

Adenosine is also formed from SAH, which is a product of all s-adenosylmethionine-dependent transmethylation reactions (Schrader, 1983). This reaction is catalyzed by SAH hydrolase.

There are at least two pathways, which serve as a sink for ado, ie. phosphorylation and deamination. The phosphorylation of ado to AMP is catalyzed by adenosine kinase. The deamination of ado to inosine is catalyzed by adenosine deaminase (Manfredi and Holmes, 1985).

In view of this brief background on the possible sources and sinks of ado, it is interesting to examine the age-related changes in the adenine nucleotide metabolism of the developing heart in the following two sections.

3.b.ii: Quantitative and functional changes in mitochondria:

The primary role of mitochondria in the cardiac cells is to provide chemical energy in the form of ATP. Quantitative and qualitative studies suggest a decreased ability of the mitochondria to synthesize ATP with increasing age. Quantitative studies reveal an increase in number and volume of mitochondria of rat hearts during the first two

weeks of development (Olivetti et al., 1980). Results of such studies on animals above 1 month of age to adult are inconsistent. Although Tomanek and Hovanec (1981) showed a slight but statistically insignificant decrease in mitochondria-to-fiber volume ratio in hearts of 1, 4, 7, 12 and 24 month-old rats, Frenzel and Feimann (1984) showed a 36% decrease in mitochondrial size and a 10% decrease in mitochondria-to-fiber volume ratio between 6 weeks and 2 years. However, this was associated with a 40% increase in the numerical density of mitochondria. Any decrease in mitochondria/fiber volume ratio suggests a decreased ratio of energy-producing to energy-consuming capacity with increasing age.

Given the quantitative changes in mitochondria to fiber relationship, it is interesting to examine the age-related changes in mitochondrial function and ATP metabolism. Nohl (1980) studied adenine nucleotide translocase (ANT) of heart mitochondria from rats between 3 and 30 months of age. ANT is an inner mitochondrial membrane enzyme which transports cytosolic ADP into the mitochondria and ATP synthesized in mitochondrial matrix to the cytosol. This investigator showed that the activity of ANT is 40% less in a 30 month-old rat heart than a 3 month-old heart. Furthermore, he found that the endogenous adenine nucleotide pool is 25% less for the old vs the young group, essentially at the expense of ATP.

The decreased activity of ANT was not due to a decrease in the number of binding sites, since this was essentially the same in both age groups. Instead, the decreased activity of ANT was accounted for by the changes in the composition of the surrounding phospholipids of the membrane. An increase in the ratio of phosphotidylcholine to

phosphotidylethanolamine of the membrane was found in the same study. These changes in the membrane lead to decrease the fluidity of the bulk phase of the membrane lipids and decreased ADP/ATP exchange capacity of the enzyme.

Another study by Chesky et al. (1980) demonstrated that the production of ATP from phosphocreatine decreases with age. These investigators studied the activity of creatine phosphokinase (CPK) of 1, 2, 4, 6, 8, 10, 12, 14 and 20 month-old rat hearts. This enzyme catalyzes the conversion of creatine phosphate to creatine and ADP to ATP. They found a gradual decrease in CPK activity with increasing age from 2 to 20 months after an initial rise in activity from 1 to 2 months.

Victoria et al. (1981) showed a gradual decline with age in Krebs cycle function of rat hearts aged 1,3,6,10,16 and 21 months. This was manifested by decreased activity of citrate synthase, $NADP^+$ and NAD-iso-citrate dehydrogenase, and an increase in the activity of succinate dehydrogenase and NAD^+-malate dehydrogenase. Taken together, the decreased activities of ANT and CPK and the changes in Krebs cycle enzymes suggest a decreased ability to rapidly form ATP with increasing age.

3.b.iii: Changes in the adenosine-generating and adenosine-degrading enzymes.

There are only a few studies on the effects of age on the cardiac enzymes responsible for adenosine formation and its degradation. Awad and coworkers (Awad and Clay, 1982; Chttopadhyay and Awad, 1983) studied cardiac sarcolemmal bound 5'nucleotidase (5' NT) in rats of 1,6 to 8 and

13 to 15 months of age. These authors found that the specific activity of this enzyme is higher for 1 vs 6 to 8 month-old hearts; and the activity did not change after this age. Kinetic studies showed that the Km value increases and the Vmax decreases with age. This was associated with a 15 to 20% decrease in the membrane polyunsaturated fatty acids, which the authors believe might account for the decreased activity of this enzyme.

More recently DeTata et al. (1985) studied the activity of 5'NT and ADA in whole homogenates of hearts of 2, 4, 8 and 52 weeks-old rats. These authors reported that 5'NT activity was unchanged up to 4 weeks (18 U), decreased to 11 U at 8 weeks, and increased again to 14 U by 52 weeks. On the other hand, ADA activity was about 9.8 U up to 4 weeks, then declined to 6.4 U at 8 and 52 weeks of age. There is more decline in ADA than in 5' NT activity. Furthermore, these investigators showed that the activities of these two enzymes vary in a graded fashion across the myocardium from the endocardium to the epicardium at all ages. The activity of the 5'NT was highest at the two edges of the myocardial wall and lowest at its midportion. In contrast ADA activity was lowest at the two edges and highest at the midportion.

A final conclusion cannot be drawn on how these changes in nucleotide metabolism would affect ado formation. There are two mechanisms which favor increased tissue Ado concentration; first, the decreased activity of ADA which slows down the rate of ado degradation, and second, the decreased ability to synthesize ATP, which favors the accumulation of the substrate AMP for 5' NT (Worku and Newby, 1983). On the other hand, the decline in 5'NT activity with age, suggests a decline in the rate of ado formation.

SIGNIFICANCE

The vascular density of the myocardium is of great importance in maintaining an adequate blood supply for myocardial perfusion. An increase in myocardial metabolism is always coupled with an increase in coronary blood flow and O_2 delivery to meet the increasing demands for O_2 utilization. However, the degree of coronary vasodilation is largely determined by the magnitude of myocardial vascularity and the dilator capacity of the resistance vessels.

Although the number of capillaries actually does increase with development, a decline in capillary density and capillary surface area occurs as a result of increased fiber diameter. In addition, the developmental process is also associated with a decreased ability of the vascular smooth muscle cells to relax in response to various vasodilating agents. Based on these findings, one might predict that the maxium coronary conductance per mass would similarly decline with age.

Adenosine is proposed to mediate the increased coronary conductance during increased myocardial metabolism. One of the physiological signals for ado formation is a decrease in the O_2 supply-to-demand ratio (Berne, 1964). This hypothesis predicts that ado release is stimulated whenever this ratio falls. Thus ado release can be viewed as a marker of relative local ischemia. If O_2 supply to an old heart is lower than that to a young one at the same level of O_2 demand, then it would be expected that the release of ado from the older heart will exceed that from the young one at the same level of myocardial metabolism. However, this prediction is complicated by the fact that 5' NT activity decreases with age, which suggests a decreased ability for ado formation by the

cardiac myocytes. In contrast, decreased ADA activity and increase accumulation of the substrates for 5' NT with age, suggest an increased ability for ado formation.

The consequences of these morphological changes in vascularity, the decreased ability of vascular smooth muscle cells to relax in response to various vasodilating agents, and the changes in adenine nucleotide metabolism, on myocardial perfusion have not been investigated. In view of these age-related changes in the mammalian heart, the current study was designed to evaluate the coronary vasodilator reserve and ado release in the developing guinea pig heart. The coronary reserve was assessed both pharmacologically and metabolically. The results of these studies are presented in the following two sections.

CHAPTER III.

EFFECTS OF DEVELOPMENT ON CORONARY VASODILATOR RESERVE IN THE ISOLATED GUINEA PIG HEART

SUMMARY

Morphological studies have demonstrated an age-related decrease in capillary density and capillary surface area in the developing heart. However, the consequences of these changes on myocardial perfusion are not known. We tested the hypothesis that the decreased capillary density is associated with a reduction in coronary blood flow reserve. To test this hypothesis, we studied coronary 1) responses to adenosine and sodium nitroprusside administration, 2) reactive hyperemia, and 3) autoregulatory capacity. We used a Langendorff-perfused heart preparation from guinea pigs of five different age groups (1 week, 1,2, 12 and 18 months). Data are expressed as mean $\pm$ SEM. Maximal coronary flows ($ml/min.g^{-1}$) in response to adenosine (10^{-6} to 10^{-5} M) infusion are: 27 ± 1.3, 18.5 ± 1.4, 12.2 ± 0.4, 10.3 ± 0.3 and 10.6 ± 0.8 at 1 week, 1, 2, 12 and 18 months, respectively, with the flows at 1 week and 1 month significantly higher than those at 2, 12 and 18 months. There is a similar trend for a decreased maximal coronary perfusion in response to sodium nitroprusside (10^{-6} to 10^{-5}M) and following a 45 second occlusion of the coronary inlet flow. Despite the decreased maximal pharmacologic and reactive hyperemic flow reserve, autoregulation of flow is not altered with growth. The pressure-flow relationship exhibits autoregulation between 25 and 55 mmHg perfusion pressure for all but the 1 week age group which autoregulates within a

narrower range of pressures (20-45 mmHg). Total maximal coronary flow (ml/min) increases during development; this indicates that the growth of vessels continues with development. However, since coronary perfusion, corrected per unit cardiac mass, significantly decreases, we conclude that the vascular growth lags behind that of the parenchyma.

INTRODUCTION:

Morphological changes in the myocardial vasculature occur during growth from neonate to adult. As a result of vascular growth, a gradual decline in fiber to capillary ratio has been reported to occur in several species of experimental animals and in human beings (Wearn, 1940; Roberts and Wearn, 1941; Rakusan et al., 1965; Tomanek, 1970; Olivetti et al., 1980; Tomanek and Hovanec, 1981). The decline continues from prenatal life until adulthood, after which the ratio remains at about 1:1. However, because of hypertrophy of myocardial fibers during development, there is an age-related fall in capillary density and capillary surface area (Roberts and Wearn, 1941, Wearn, 1941; Rakusan et al., 1967; Tomanek, 1970; Henquell et al., 1976; Lund & Tomanek, 1978, Tomanek and Hovanec, 1981; Tomanek et al., 1982).

The consequences of these morphological changes on coronary perfusion have not been studied. In fact, little is known about the influence of development on the maintenance of myocardial perfusion. There are no reports of attempts to correlate the age-related morphological changes in the growing heart with changes in coronary perfusion.

We tested the hypothesis that the decreased capillary density reported to occur early in development is associated with a decreased coronary blood flow reserve. To test this hypothesis we studied the influence of age on coronary 1) responses to adenosine and sodium nitroprusside administration 2) reactive hyperemia, and 3) autoregulatory capacity.

MATERIALS AND METHODS:

Guinea pigs of five different age groups (1 week and 1,2,12 and 18 months) were studied. Following anesthesia (sodium pentobarbital: 50 mg/kg, ip), their hearts were rapidly excised and immersed in ice-cold physiologic salt solution (PSS) (NaCl: 117 mM; KCl: 4.7 mM; KH_2PO_4: 1.2 mM; $MgSO_4$: 1.2 mM; $NaHCO_3$: 21 mM; Na_2EDTA: 0.2 mM; $CaCl_2$: 2.7 mM; glucose: 8 mM; pyruvate: 2 mM). The aorta was cannulated and the heart perfused in a retrograde fashion. All hearts were maintained in a nonworking state by venting the left ventricle with a catheter through the mitral valve. The hearts were electrically paced at 300 beats/minute. The PSS was equilibrated with 95% oxygen + 5% carbon dioxide, continuously filtered through a 3 um filter, maintained at 37°C and pH 7.4, and not recirculated. Perfusion pressure was maintained constant at 46 mmHg. Perfusate PO_2 was determined by using a Corning blood gas analyzer before drug infusion and periodically throughout the experiment to insure adequate oxygenation of the perfusate. Coronary flow was determined by means of an electromagnetic flow probe in the aortic line or by timed collection of the coronary venous

effluent. Coronary flow was expressed as ml/min/g wet heart weight. Heart weight was measured after trimming away the great vessels and fat, and blotting with filter paper.

PROTOCOL:

Control flow was measured after a 40 minute equilibration period. The maximum reactive hyperemic response was measured following a 45 second occlusion of the inlet flow into each heart of the five age groups. In the first two sets of experiments the dose-response relationship between coronary flow and adenosine or sodium nitroprusside was established for the five age groups. Drugs were infused into the aortic cannula at a rate of 0.1 ml/min. This was done over a range of 10^{-9} to 10^{-5} M perfusate concentrations. Maximal flow was measured and the concentration producing the half maximal response (ED_{50}) was calculated for each drug infused in each age group. Half maximal flow was calculated in the following way: control flow + 1/2 (maximum flow - control flow). The dose producing this flow (i.e. ED_{50}) was then assessed by interpolation to the dose which produced the half maximum flow on the dose-response curves. In a third set of experiments nonpaced hearts from 1 week and 1 and 18 month-old guinea pigs (n=4) were arrested with a high dose of adenosine (5×10^{-5}M). Coronary flow was then measured in the absence of extravascular wall compression. In the fourth set of experiments the autoregulatory capacity in 4 age groups (1 week, 1, 12 and 18 months old) was assessed by measuring steady-state coronary flow following 5 mmHg step-changes in perfusion pressure within a

range of 20-80 mmHg. The gains for the autoregulatory curves were calculated according to the following formula: $G = [\ \Delta F/F \div \Delta P/P\ ] - 1$ (Morff and Granger, 1983). Where G = gain, F = coronary flow, P = perfusion pressure, and ΔF and ΔP represent the difference between the final and the initial flow and pressure, respectively.

STATISTICAL ANALYSIS:

Results are expressed as mean $\pm$ SEM. We used one-way, between-group analysis of variance and Student-Neuman-Keuls tests for multiple comparisons between mean data (Linton and Gallo, 1975) to compare values for maximum coronary flow, ED_{50}, body weight, heart weight, heart weight-to-body weight ratio and myocardial water content in the 5 age groups. The same tests were used to compare coronary flows or gains among the different age groups at various perfusion pressures. One-way, within-group analysis of variance and Student-Neuman-Keuls tests were applied to the pressure-flow or pressure-gain data to determine the autoregulatory range for each age-group. Differences between mean data were considered significant at $p<0.05$.

RESULTS:

Relationship between body weight and heart weight. The data pertaining to the heart weight, body weight and myocardial water

content for each of the five age groups are summarized in Table 1. Heart weight and body weight almost doubled between 1 week and 1 month, 1 and 2 months, and 2 and 12 months. The weights did not change after 12 months. The heart weight-to-body weight ratio was similar between 1 week and 1 month old guinea pigs. However, the ratio in these two groups was significantly higher than the other three age groups. The ratio was not different between 12 and 18 month old guinea pigs, however, both were significantly lower than the 2 month old group. The differences in heart weight were not due to differences in water content.

Reactivity of the coronary bed to pharmacologic stimuli. The dose-response relationship of coronary flow (ml/min/g) to adenosine infusion is presented in Figure 1. Maximal coronary flows were achieved at concentrations of 10^{-6} to 10^{-5} M in all age groups. The maximal coronary flow per gram (Table 2) at 1 week was significantly higher than at 1 month of age. In addition, the maximal flow in these two groups was significantly higher than at 2, 12 and 18 months. No significant differences were observed among the 2, 12 and 18 month old groups.

To test whether the decreased vasodilator capacity is specific to adenosine, the effect of sodium nitroprusside was studied in another set of experiments (Figure 2). A similar trend for an age-related decline in maximal coronary flow was observed in response to sodium nitroprusside (Table 2).

Despite the decreased responsiveness to adenosine and sodium nitroprusside in 2, 12 and 18 month hearts, there were no significant differences in the ED_{50} values for these 2 drugs among the five age groups (Table 2).

To test whether the age-related decrease in vasodilator reserve is specific to pharmacologic stimuli, we studied the effect of development on coronary reactive hyperemic responses. As in the pharmacologic hyperemic responses, a similar age-related decline in vasodilator capacity was observed with reactive hyperemic responses (Table 3).

To test the influence of extravascular compression on coronary vasodilator reserve, a high dose of adenosine (5×10^{-5}M) was infused into non-paced hearts. This dose of adenosine simultaneously arrests the heart probably by causing atrio-ventricular block (Bellardinelli et. al., 1982) and a maximum vasodilator response. Coronary flows were 35.9 ± 0.7, 24.2 ± 0.4 and 16.8 ± 0.5 ml/min/g for 1 week, and 1 and 18 month-old hearts, respectively. Thus, the age-related decrease in maximum coronary flow between 1 week vs 1 month and 1 vs 18 months is still demonstrated ($p<0.05$) despite the elimination of extravascular compression.

The maximum total coronary flows (ml/min) of each of the five age groups were compared in Figure 3. Total flows in 12 and 18 month-old hearts were similar and both are significantly different from the other three groups. Total flow at 1 week of age is significantly lower than at 1 and 2 months of age. Despite the decreased coronary flow per unit mass in the 2 month

old group vs 1 month group (Figures 1 and 2), no significant differences were observed in the total flows for the two age groups (Figure 3).

Table 1. Effect of development on body weight, heart weight, heart weight-to-body weight ratio and percent water content of 1 week and 1, 2, 12 and 18 month-old guinea pigs.

	AGE				
	1 week	1 month	2 months	12 months	18 months
Body weight (g)	124* ±4	272** ±9	521*** ±12	1064 ±27	1105 ±25
Wet heart Weight (g)	0.46* ±0.01	0.96** ±0.03	1.68*** ±0.07	2.92 ±0.09	3.02 +0.08
Heart weight/ Body weight ($x10^3$)	3.73** ±0.01	3.55** ±0.08	3.22*** ±0.08	2.74 ±0.04	2.74 ±0.06
%water content of hearts	82.1 +0.3	83.9 ±0.6	83.2 ±0.5	82.2 ±0.4	83.0 ±0.3

Values are presented as mean ± SEM (n=12)

* $p<0.05$ vs 1, 2, 12 and 18 month old group

** $p<0.05$ vs 2, 12 and 18 month old group

*** $p<0.05$ vs 12 and 18 month old group

Figure 1: The steady-state coronary flow responses to various perfusate adenosine concentrations. * $P < 0.05$ vs 2, 12 and 18 month maximum coronary flow. ** $P < 0.05$ vs respective maximum coronary flow values. Values are presented as means $\pm$ SEM (n=6). When the SEM bars are not shown, they are smaller than the size of the symbols.

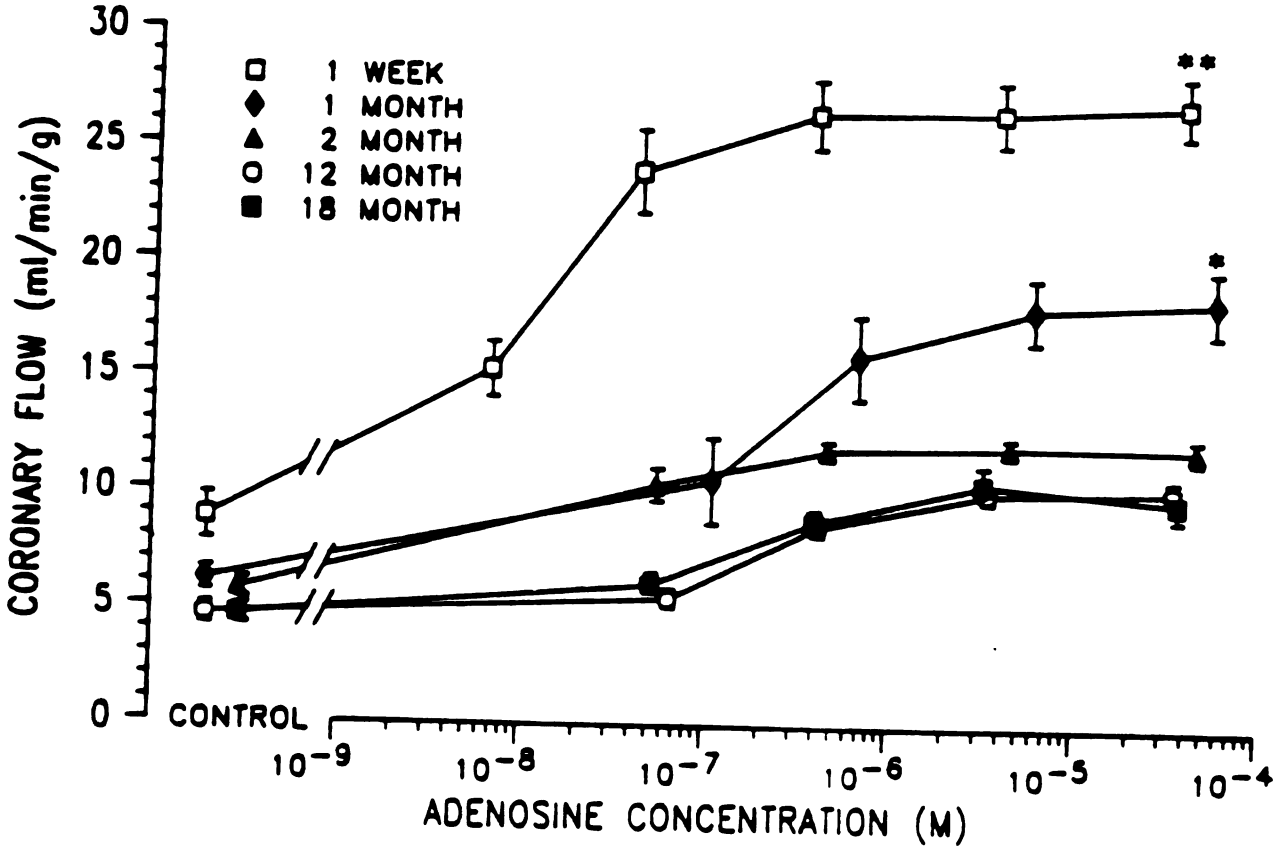
30
25
20
15
10
5
0
CORONARY FLOW (ml/min/g)
1 WEEK
1 MONTH
2 MONTH
12 MONTH
18 MONTH
**
*
CONTROL
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
ADENOSINE CONCENTRATION (M)

Figure 2: This figure shows steady-state coronary flow responses to sodium nitroprusside infusion which resulted in the perfusate concentrations shown on the abscissa. Values represent the mean $\pm$ SEM (n=6). * $P < 0.05$ vs 2, 12 and 18 month maximum coronary flows. ** $P < 0.05$ vs respective maximum coronary flow values. Where the SEM bars are not shown, they are smaller than the size of the symbols.

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Values

2, 12

0.05 vs

the SEM

size of

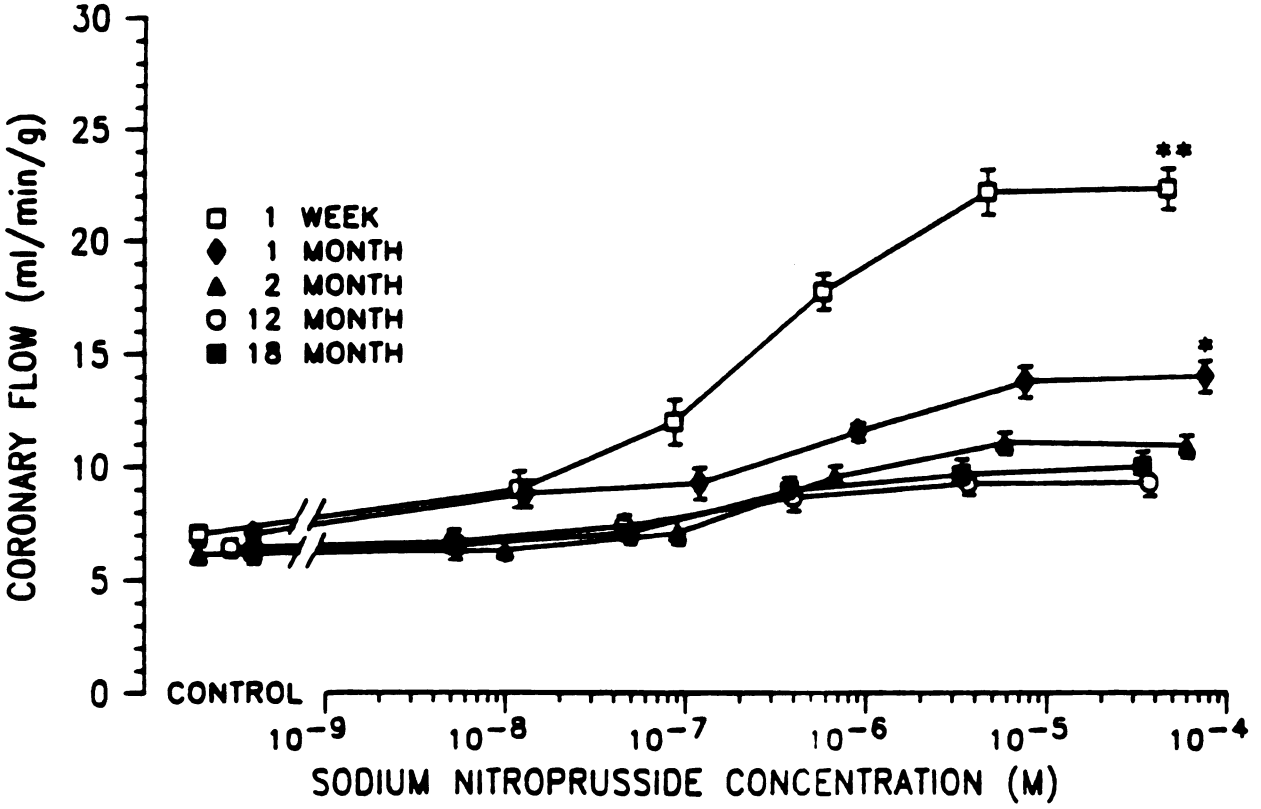


Figure 3: Illustrated here are total maximum coronary flows (ml/min) in response to adenosine (10^{-5}M) or sodium nitroprusside (10^{-5}M) for 1 week, 1, 2, 12 and 18 month age groups. * $P < 0.05$ vs 1 wk, 1 mo and 2 mo groups, ** $P < 0.05$ vs 1 wk group, + $P < 0.05$ vs 12 or 18 month difference in maximum flow between adenosine and nitroprusside. Values represent mean $\pm$ SEM (n=6).

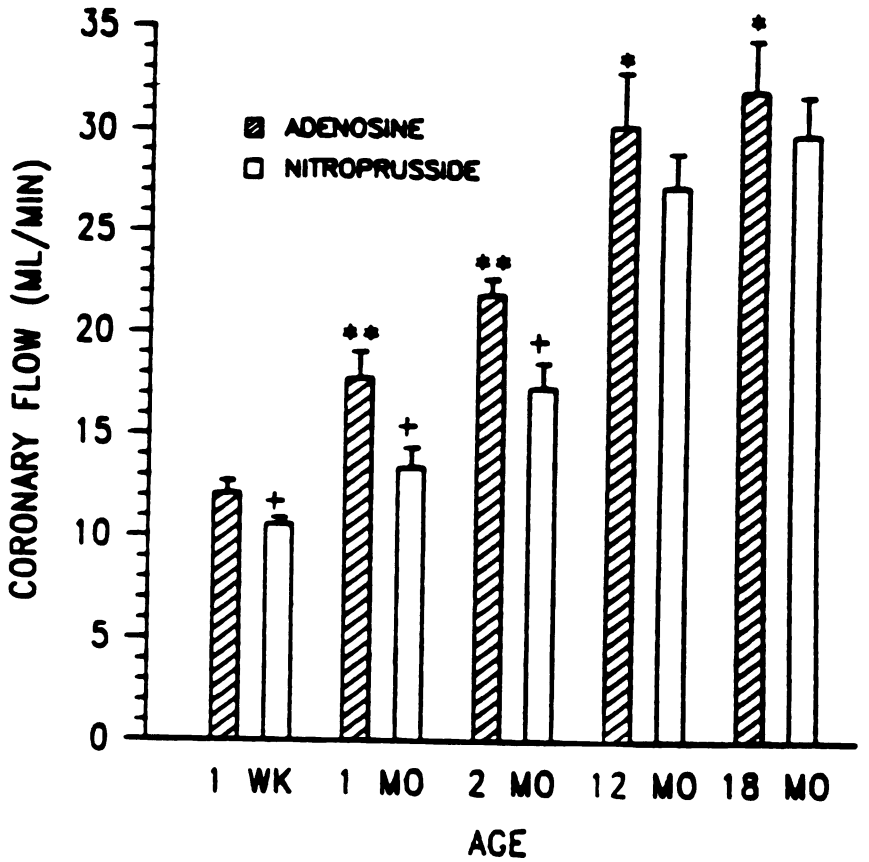

35
30
25
20
15
10
5
0
CORONARY FLOW (ML/MIN)
ADENOSINE
NITROPRUSSIDE
1 WK
1 MO
2 MO
12 MO
18 MO
AGE

Table 2. Maximum coronary flow (ml/min/g) and ED_{50} values (M) for adenosine and sodium nitroprusside in five age groups of guinea pig hearts.

	AGE				
	1 week	1 month	2 months	12 months	18 months
ADENOSINE					
Maximum coronary flow	27.0* ±1.3	18.5** ±1.4	12.2 ±0.4	10.3 ±0.3	10.6 ±0.8
ED_{50}	1.7×10^{-8} $\pm6.9\times10^{-9}$	2.6×10^{-7} $\pm7.9\times10^{-8}$	4.5×10^{-8} $\pm1.5\times10^{-8}$	2.5×10^{-7} $\pm2.6\times10^{-8}$	2.0×10^{-7} $\pm9.2\times10^{-8}$
NITROPRUSSIDE					
Maximum coronary flow	22.5* ±1.0	14.0** ±0.7	11.1 ±0.5	9.3 ±0.6	10.0 ±0.7
ED_{50}	2.0×10^{-7} $\pm4.8\times10^{-8}$	4.8×10^{-7} $\pm2.3\times10^{-7}$	3.2×10^{-7} $\pm4.7\times10^{-8}$	1.7×10^{-7} $\pm7.4\times10^{-8}$	1.8×10^{-7} $\pm2.0\times10^{-8}$

Values are mean ±SEM (n=6)

* $p<0.05$ vs 1, 2, 12 and 18 month old group

** $p<0.05$ vs 2, 12 and 18 month old group

Mean ED_{50} values for adenosine and sodium nitroprusside in different age groups are not significantly different.

Reactivity of coronary bed to changes in perfusion pressure: The results of experiments in which coronary perfusion pressure was systematically altered between 20 and 80 mmHg are shown in Figure 4. Under steady-state conditions the pressure-flow relationships exhibit autoregulation between 25 and 55 mmHg for all but the 1 week age group which regulates within a narrower range of pressures (20 to 45 mmHg). The pressure-flow curves were not significantly different among the 1,12 and 18 month age groups at any given point. However, in the 1 week group, coronary flow was significantly higher than the other three groups at any given point. In order to demonstrate more clearly the effect of development on the autoregulatory capacity, the gains for the curves were calculated. Figure 5 shows plots of gain vs. pressure for the four age groups. The negative values for gain indicate autoregulation; positive values indicate a dominance of passive elasticity over autoregulation. The autoregulatory gains for the 1, 12 and 18 month age groups were not significantly different at any given pressure; however, they were significantly different from the one week age group at 35, 50, 55, 60 and 65 mmHg. Furthermore, the one week-old group apparently autoregulates over a narrower range; they don't autoregulate as well at higher perfusion pressures as do the other three groups.

Figure 4: This figure depicts the pressure-flow relationships in guinea pig hearts for 1 week, 1, 12 and 18 month age groups. Steady-state coronary flow was determined following 5 mmHg step changes in perfusion pressure. Data for coronary flow are expressed as mean $\pm$ SEM (n=6). Coronary flow did not significantly differ between 25 and 55 mmHg for 1, 12 and 18 month age groups. For the one week-old group, coronary flow was not different between 20 and 45 mmHg. Coronary flow for one week-old hearts is significantly higher than the older groups at any given pressure. When the SEM bars are not shown, they are smaller than the size of the symbols.

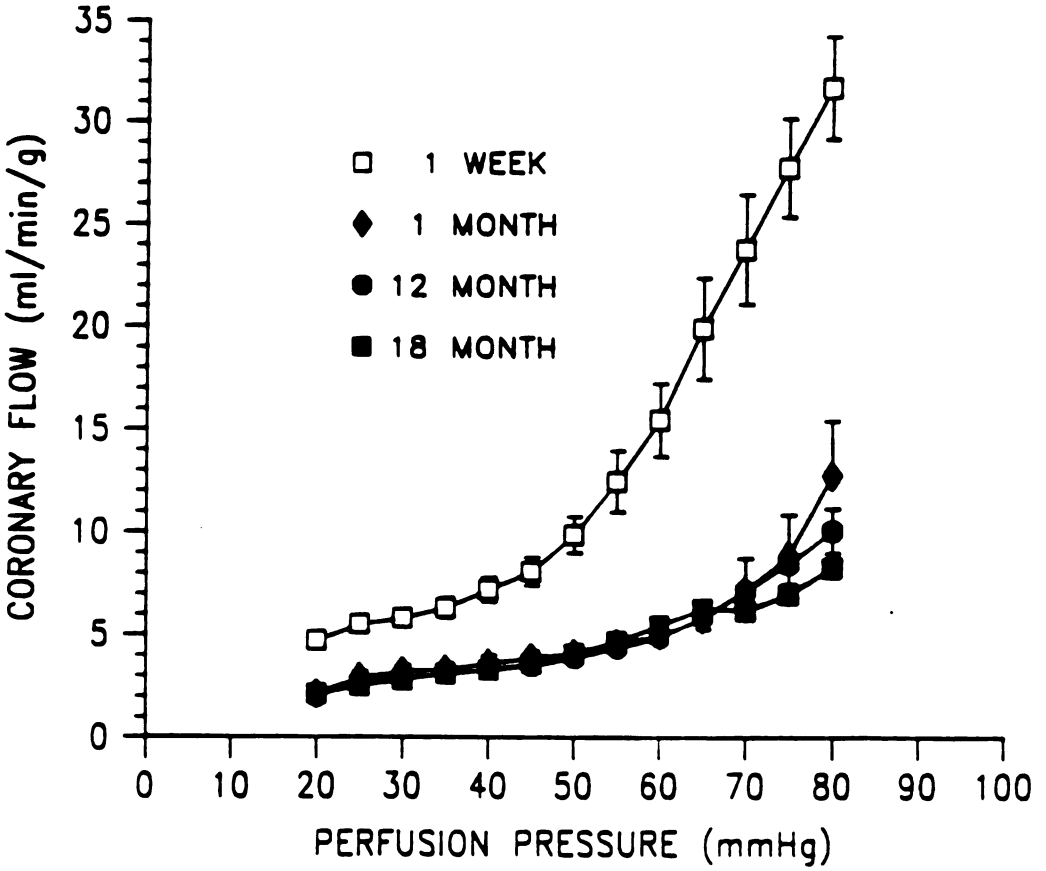

CORONARY FLOW (ml/min/g)
35
30
25
20
15
10
5
0
1 WEEK
1 MONTH
12 MONTH
18 MONTH
0
10
20
30
40
50
60
70
80
90
100
PERFUSION PRESSURE (mmHg)

Figure 5: Shown are pressure-gain relationships in guinea pig hearts of 1 week, 1, 12 and 18 months of age. Symbols represent the mean values for 1 week and 12 and 18 month groups. The shaded area represents the SEM for the one month-old group (n=6). The gains for 1 week-old hearts differ significantly from the other age groups at perfusion pressures of 35, 50, 55, 60 and 65 mmHg. The gain (G) is calculated according to the following equation (Morff and Granger, 1983):

$$G = [\Delta F/F \div \Delta P/P] - 1$$

where F = coronary flow, P = perfusion pressure, ΔF and ΔP represent the difference between the final and initial values.

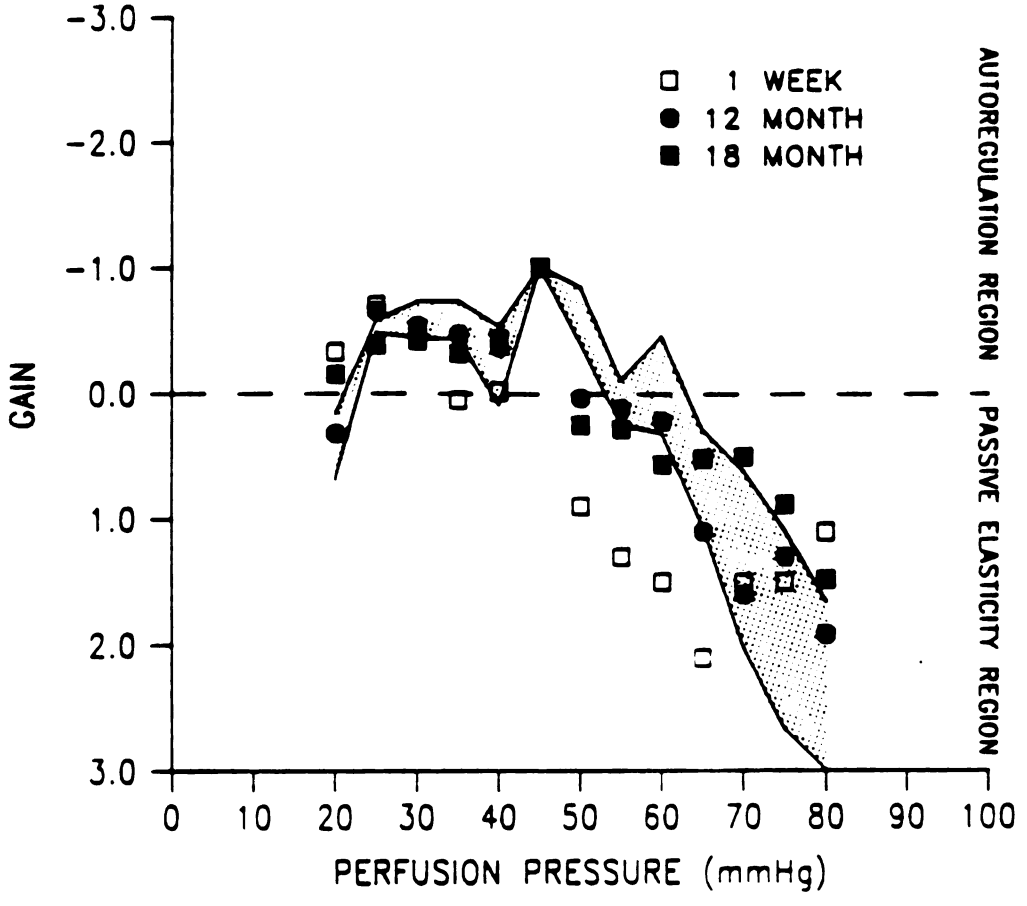

-3.0
-2.0
-1.0
0.0
1.0
2.0
3.0
GAIN
1 WEEK
12 MONTH
18 MONTH
AUTOREGULATION REGION
PASSIVE ELASTICITY REGION
0
10
20
30
40
50
60
70
80
90
100
PERFUSION PRESSURE (mmHg)

Table 3. Maximum coronary flow (ml/min/g) in response to adenosine (ADO), sodium nitroprusside (SNP) and reactive hyperemia (RH) in guinea pig hearts of five age groups.

AGE	Maximum coronary flow (ml/min/g) in response to:		
	ADO	SNP	RH
1 week	27.0*	22.5*	20.1*
	±1.3	±1.0	±0.6
1 month	18.5**	14.0**	16.6**
	±1.4	±0.7	±0.5
2 months	12.2	11.1	13.4
	±0.4	±0.5	±0.2
12 months	10.3	9.3	11.4
	±0.3	±0.6	±0.5
18 months	10.6	10.0	12.2
	±0.8	±0.7	±0.6

Values are mean ± SEM (n=6)

* $p<0.05$ vs 1, 2, 12, and 18 month-old group

** $p<0.05$ vs 2, 12, and 18 month-old group

DISCUSSION:

We investigated the effects of myocardial growth and development on coronary flow reserve in guinea pig hearts. The major findings in this study can be summarized as follows:

1) Maximum coronary flow per gram heart weight was highest in the neonate and declined with heart growth until sexual maturity (2 months of age).
2) Total coronary flow increased with development, but not in proportion to heart weight until sexual maturity was achieved (Figure 3).
3) The autoregulatory capacity of the coronary bed was not altered (Figures 4 and 5).

This discussion will focus on three areas: the effect of myocardial development on the pharmacologic and physiologic vasodilator reserve, the effect of development on the autoregulatory capacity of the coronary bed, and the factors involved with alterations in vasodilator reserve.

Effect of growth on pharmacologic and reactive hyperemic responses. The different pharmacologic interventions (adenosine and sodium nitroprusside) and transient coronary occlusion resulted in different maximum coronary flow responses (Table 3). This variation could be the result of: (1) different degrees of relaxation of vascular smooth muscle, (2) different sites of action of the three stimuli (i.e. small vs large vessels) and/or (3) differences in ventricular compression. We cannot distinguish among these options. However it is important to

recognize that regardless of the quantities of maximum flow, the qualitative conclusion stands: maximum coronary flow fell with maturation. The dilator reserve demonstrated in this study is similar to that seen when myocardial blood flow is measured (microspheres) before and during maximum dilation (dipyridamole) in conscious rats (Wangler et al., 1982). The magnitude of the reactive hyperemic responses reported in this study is also similar to that elicited from intact, blood-perfused rat hearts (Peters et al., 1984) and Langendorff-perfused guinea pig hearts (Schrader et al., 1977). This study demonstrated an age-related decrement in the pharmacologic as well as reactive hyperemic responses in the guinea pig coronary bed (Table 3). Thus, it appears that the previously observed morphologic decrease in vascular density of the developing heart from the neonatal life to adulthood in humans, rabbits and rats (Wearn, 1941; Rakusan, et al., 1967; Tomanek et al., 1982) is associated with a diminishing maximal coronary flow response in the guinea pigs reported in this study.

The maximal total coronary flow not corrected for cardiac mass can be viewed as an index of the total vascular conductance (Mueller et al., 1978). Between the ages of 1 week and 1 month total flow increases significantly (Figure 3), cardiac mass doubles (Table 1) and myocardial perfusion (defined here as flow/gram) decreases significantly (Figures 1 and 2; Table 3). At 1 and 2 months, total coronary flow is similar, in spite of a doubling of cardiac mass, and again perfusion declines significantly. These results indicate that the growth of coronary

bed lags behind parenchymal growth. Total coronary flow at 12 or 18 months significantly exceeds that at 2 months. However, if flow is corrected for heart mass, the perfusion does not differ among these three ages in spite of a doubling of cardiac mass between 2 and 12 or 18 months. These data indicate that a relative slowing of the parenchymal growth rate between 2 and 12 months enabled the proliferating vasculature to keep pace.

We conclude that vascular proliferation continues with growth of the heart; initially, however, it is not proportional to the parenchymal growth rate.

Effect of age on coronary autoregulation. The intrinsic ability of the isolated heart preparation to regulate coronary flow over a wide range of perfusion pressures has been demonstrated in several studies (Bunger et al., 1975; Schrader et al., 1977; Bardenheuer and Schrader, 1983). The pressure-flow relationship in this study demonstrated autoregulation between 25 and 55 mmHg in 1, 12 and 18 month-old guinea pig hearts (Figure 4); this range compares favorably with the previously cited studies. However, the one-week old guinea pig hearts do not appear to autoregulate as well at higher perfusion pressures. These observations were confirmed after calculating the gains for the pressure-flow responses (Figure 5). It appears that autoregulatory capacity in the one week-old hearts is impaired; this is demonstrated by values of gain at pressures of 35, 50, 55, 60, and 65 mmHg, which are significantly different from the three older groups in the positive direction. As perfusion

pressure increases, autoregulatory capacity is exhausted earlier and coronary flow is modulated predominately by passive coronary elasticity. On the other hand, lowering perfusion pressure to 20 mmHg resulted in similar coronary flow responses for 1, 12, and 18 month age groups. This indicates that, despite previously reported age-related decreases in capillary density (Rakusan, 1967; Tomanek et al., 1982) and our observation of decreased pharmacologic and reactive hyperemic responses, the developmental process does not affect the usable autoregulatory reserve of the coronary bed. Infusion of adenosine at various points on the descending limb of the autoregulation curve of dog hearts results in a further decrease in coronary vascular resistance (Sparks et al., 1984) demonstrating that resistance vessels do not maximally dilate in response to lowered perfusion pressure. If reduced perfusion pressure does not elicit maximal vasodilation in isolated guinea pig heart, this could explain the apparent discrepancy between the pharmacologic and autoregulatory responses.

Factors involved in the decreased vasodilator reserve. There are several explanations which could account for the age-related decrement in coronary flow reserve. One possibility is an increase in extravascular pressure secondary to an age-related increase in myocardial stiffness. This possibility is unlikely because increased myocardial stiffness is associated with senescence (Yin et al., 1980) and we have demonstrated a decrease in the dilator reserve as early as 1 month of age. In fact, the

data obtained from arrested hearts demonstrate that differences in flow remained despite the elimination of intramyocardial compressive effects. Decreased coronary dilator capacity could result from increased stiffness of vessel wall with age due to increased connective tissue deposition which limits the ability of the coronary vessel to dilate. This is unlikely because coronary vessels of 1 week, 1, 12 and 18 month hearts show comparable degrees of passive elasticity judging from the increase in flow in response to high perfusion pressures (Figure 5). The diminished responsiveness to vasodilation is not the result of down regulation of a specific receptor because adenosine, sodium nitroprusside and reactive hyperemia are all decreased. It is possible that the decreased flow reserve is due to impaired relaxation of vascular smooth muscle. The decreased relaxation of aortic strips in response to isoproterenol (Fleisch et al., 1970; Fleisch and Hooker, 1976) dopamine (Cohen and Berkowitz, 1975) and cyclic AMP (Cohen and Berkowitz, 1974) has been related to impaired relaxation mechanism during aging. Numerous studies have shown an age-related increase in stiffness of the myocardium (Tempelton et al., 1979; Yin et al., 1980; Spurgeon et al., 1983). These changes have been attributed to impaired sarcoplasmic reticular handling of Ca^{++} ions (Froehlich et al, 1978). It is possible that a similar age-related impairment in relaxation of coronary vascular smooth muscle could account for the decreased pharmacologic dilator reserve reported in this study. Finally, the decreased reserve could be the result of decreased vascular density. Several studies on cardiac

hypertrophy suggest that the reduction in coronary vasodilator reserve (reflected by increased minimum coronary vascular resistance) is at least partially the result of failure of total cross-sectional area of the coronary bed to increase in proportion to increased cardiac mass (Mueller et al., 1978; Murray and Vatner, 1981; Wangler et al., 1982; Peters et al., 1984). The age-related decrement in coronary flow reserve found in this study appears to be closely associated with the reported decrement in capillary density and capillary surface area during cardiac development of humans, rabbits and rats (Wearn, 1941; Rakusan et al., 1967; Tomanek et al., 1982). Currently, available information is not adequate to determine if the increased resistance is the result of decreased capillary density or a parallel decrease in another vascular segment such as the arterioles. Adenosine and nitrites have a preferential dilator action on small and large coronary arteries, respectively (Schnaar and Sparks, 1972). As depicted in Figure 3, the difference between total maximum coronary flow in response to adenosine vs. nitroprusside is significantly different at 1 week, 1 month and 2 months. Therefore, we speculate that there is a tendency for development to affect the small resistance vessels preferentially to larger arterioles.

In summary, the maturational process is associated with a decreased coronary flow reserve in the guinea pig heart, although it does not seem to affect its capacity for autoregulation. Although the possibility of an impaired relaxation mechanism as a

cause of this decrement has not been ruled out, we think that failure of the coronary bed to keep pace with myocardial growth is the most likely reason for the decline in flow reserve.

CHAPTER IV

CORONARY FLOW, MYOCARDIAL O_2 CONSUMPTION AND ADENOSINE RELEASE IN YOUNG AND ADULT GUINEA PIG HEART.

Summary:

Myocardial growth is accompanied by a decrease in vascular density. Previous studies from our laboratory indicate an age-related decrease in coronary vasodilator reserve elicited pharmacologically and by transient coronary occlusion (Toma et al., Circ. Res. 57:538-544, 1985). The current study was designed to examine the influence of age on coronary metabolic vasodilation, myocardial O_2 consumption ($\dot{M}VO_2$) and adenosine release (RADO) during norepinephrine (NE) infusion. Hearts from 1 month (young) and 18 month (adult) guinea pigs (n=6) were isolated and Langendorff-perfused with physiologic salt solution. NE was infused (0.1 ml/min) over a range of $1x10^{-8}$ to $5\ x10^{-6}$ M perfusate concentration. Maximum coronary flow [CF (ml/min/g)] and oxygen delivery [$\dot{D}O_2$ (ulO_2/g/min)] are (mean±SEM) 11.9±0.4 vs 7.6±0.4 and 210.0±7.4 vs 141.0±7.4 for 1 vs 18 month-old hearts, respectively. Maximum values for $\dot{M}VO_2$ (ulO_2/g/min) are 185.0+5.0 vs 135.0±9.0 in 1 and 18 month-old hearts, respectively. Maximum peak and steady-state RADO (nmol/g/min) were similar: 9.086±1.969 vs 7.414±0.796 and 2.158±0.583 vs 2.496±0.176 for young vs adult hearts, respectively. At a given $\dot{M}VO_2$, RADO is significantly higher for the 18 month-old hearts when compared to the 1 month-old group. However, when RADO is plotted against venous PO_2, RADO is higher in

the young than in the adult hearts. Venous oxygen tension of the 18 month-old heart was significantly lower than for 1 month-old group during NE infusion (49.0±7.0 vs 90.0±7.0 mmHg). In the 18 month-old heart, CF is similar at a given MVO_2 when MVO_2 is raised either by isoproterenol (ISO) or NE. Data from this study show that 1) at a given MVO_2, CF and DO_2 are significantly lower for the adult hearts when compared to the young hearts, 2) RADO and O_2 extraction are significantly augmented for 18 vs 1 month-old hearts, at the same MVO_2, and 3) the increased RADO in the adult hearts is not related to their lower tissue PO_2. We conclude that 1) coronary flow reserve is decreased with age during NE stimulation, 2) this is not the result of an enhanced alpha-adrenergic activity with age, and 3) the adult heart may compensate for the decline in CF and DO_2 by increasing O_2 extraction and increasing RADO to achieve further vasodilation.

INTRODUCTION

A fall in myocardial fiber-to-capillary ratio with maturation from neonatal life to adulthood, reflects capillary proliferation in the developing mammalian heart (Wearn, 1940, Rakusan et al, 1965). Despite the increased number of capillaries, increased myocardial fiber diameter results in a consistent fall in vascular density and vascular cross-sectional area, per unit tissue area (Rakusan et al., 1967; Tomanek et al., 1982) and an increase in coronary intercapillary distance for the diffusion of oxygen (Henquell et al., 1976).

We have recently demonstrated that these morphologic changes affect myocardial perfusion. The vasodilator capacity of the developing

guinea pig heart is decreased in response to adenosine, sodium nitroprusside and transient coronary occlusion (Toma et al., 1983 and 1985). The time-course for the reduction in vasodilator reserve parallels the decline in vascular density (Tomanek et al., 1982). Coronary reserve is highest in the neonate (one week), declines with cardiac growth until the age of maturity (2 months) and stays the same up to middle age (18 months). The consequences of the decreased coronary vasodilator reserve on myocardal perfusion during metabolic stimulation have not been studied.

We tested the hypothesis that the age-related decrease in coronary vasodilator reserve, described above, is associated with a decreased coronary dilator capacity during metabolic stimulation. To test this hypothesis we studied the influence of age on the response of coronary flow and myocardial oxygen consumption to norepinephrine. In preliminary experiments we found that coronary flow was depressed for a given myocardial oxygen consumption ($\dot{M}VO_2$) in 18 vs 1 month-old guinea pigs. Therefore, we also tested the hypothesis that the lower coronary flow at a given $\dot{M}VO_2$ would result in a greater release of adenosine.

MATERIALS AND METHODS

Hearts from 1 and 18 month-old guinea pigs were studied. Animals were stunned by a blow to the head. Their hearts were rapidly excised and immersed in ice-cold physiologic salt solution (PSS), containing: NaCl, 117 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 21 mM; glucose,8 mM; $CaCL_2$, 2.2 MM; and pyruvate, 2mM. The aorta was cannulated and the heart was perfused via retrograde aortic perfusion with the PSS. The PSS was equilibrated with 95% oxygen and 5% carbon

dioxide, maintained at 37° C and pH 7.4, and not recirculated. Perfusion pressure was maintained constant at 46 mmHg. The hearts were maintained in a non-working state by venting the left ventricle with a catheter through the mitral valve. A pressure transducer was placed at a point in the arterial cannula about 2 cm above the heart to monitor perfusion pressure and heart rate from the pressure tracing. Coronary flow was measured by means of an electromagnetic flow probe in the aortic line and was expressed as ml/min/g wet heart weight. The heart was weighed after trimming away the great vessels and fat, and blotting with filter paper. The pulmonary artery was cannulated for collection of the coronary venous effluent. The effluent partial pressure of oxygen (PO_2) was measured either by sampling the pulmonary artery effluent for determination with a blood gas analyzer or via an indwelling oxygen electrode (Instech Laboratories, Oxygen Uptake System 102 A). Flow, pressure and venous PO_2 were continuously recorded on a Grass Polygraph. The indwelling O_2 electrode was calibrated by direct measurement of effluent P_{O2} with a Corning blood gas analyzer.

Myocardial oxygen consumption ($\dot{M}VO_2$) was expressed as ul O_2/min/gram and was calculated according to the equation: $\dot{M}VO_2 = CF \times (Pa - Pv) \times (C/760)$, where CF = coronary flow (ml/min/gram wet weight), Pa - Pv = perfusate minus coronary venous effluent difference in PO_2 (mm Hg), and C = Bunsen solubility coefficient of oxygen dissolved in perfusate at 37° (23 ul O_2/atm/ml perfusate) (Zander and Euler, 1976). Delivery of O_2 ($\dot{D}O_2$) was calclated as

$$\dot{D}O_2 = CF \times PaO_2 \times C/760$$

The release of adenosine (pmol/min/gram) was calculated as the product of the effluent concentration and the coronary flow.

Protocol:

All hearts were allowed to equilibrate for a period of 30-40 minutes after establishing retrograde perfusion. When steady-state coronary flow was obtained, the reactivity of the coronary bed to transient occlusion was tested. Hearts with reactive hyperemic responses of less than 2 times control flow were rejected.

In the first set of experiments the dose-response relationship between CF or $\dot{M}VO_2$ and norepinephrine was established for the two age groups. Norepinephrine (NE) was infused into the aortic cannula over a range of 1×10^{-8} to 5×10^{-6}M perfusate concentrations. Maximum CF and MVO_2 were measured and the dose producing the half maximum response (ED_{50}) was calculated for each age group. Half maximum responses for CF and MVO_2 were calculated in the following way: control value + 1/2 (maximum response - control value). The dose producing this response (i.e. ED_{50}) was then assessed by linear interpolation to the dose which produced the half maximum response on the log dose-response curve.

In the second set of experiments, control coronary flow was measured. Samples of perfusate and coronary effluent were collected for the determination of control adenosine release in six 1 month and six 18 month-old hearts. In addition, the PO_2 of the perfusate and coronary effluent was measured in order to determine the perfusate minus venous oxygen difference. NE was then infused (at a rate of 0.1 ml/min) into the aortic cannula (1 cm above the heart) for 12 minutes. Each heart received two doses of NE: 5×10^{-8} M and 5×10^{-6} M perfusate

concentration. During the infusion of each dose, the coronary effluent was continuously collected in 30 second aliquots for the first 3 minutes. Thirty-second fractions of effluent were subsequently taken at 6, 9, and 11 minutes. Effluent fractions were then analyzed for adenosine content (see Adenosine Measurement), and adenosine release was calculated. During NE infusion, PO_2 of the pulmonary artery effluent was continuously measured via the indwelling O_2 electrode.

A third series of experiments was designed to evaluate the extent to which alpha-adrenergic activity limits oxygen delivery in the 18 month-old heart. Twelve hearts from 18 month-old guinea pigs were divided into two groups. Each heart of the first group (n=6), received six doses of isoproterenol over a range of 5×10^{-9} to 5×10^{-6} M perfusate concentration. Norepinephrine (six doses, over a range of 5×10^{-8} to 1×10^{-5} M perfusate concentration) was administered into each heart of the second group. After an initial equilibration period, control coronary flow and perfusate and pulmonary artery effluent PO_2 were measured. Steady-state coronary flow and pulmonary artery effluent PO_2 were measured for each dose during drug infusion.

Adenosine Measurement:

Samples of coronary effluent (2 ml) were evaporated and resuspended in 400 ul ultra-pure distilled water. The undissolved salts were separated by centrifugation. The supernatent was assayed for adenosine by high performance liquid chromatography (HPLC). Two pumps were programmed for gradient elution of 200 ul sample injected directly onto a reverse-phase, 5 um C-18 column. A linear gradient at a flow of 1.1 ml/min over 35 minutes was used, with initial conditions of 90% 4mM

KH_2PO_4 and 10% 70/30 methanol/water (vol/vol). Final conditions were 70% 4mM KH_2PO_4 and 30% 70/30 methanol/water (vol/vol). Absorbance of the column eluate was continuously monitored at 254 nm. Peaks were identified by comparison with retention times of external standards and were varified by intermittent duplicate samples with addition of a known concentration of adenosine or of adenosine deaminase. Adenosine was quantitated by determining the area under the optical density peak and comparing it to an adenosine standard. The above procedure resulted in >90% recovery of adenosine added to coronary effluent. We defined adenosine release as the product of coronary flow per gram and venous effluent concentration. This is a net term which reflects both production and uptake by cellular elements in the heart.

Statistical Analysis:

One-way, between group analysis of variance was used to test for significant differences in maximum CF and $\dot{M}VO_2$, and ED_{50} between the two age groups. We used linear regression analysis to find the relationship between CF and $\dot{M}VO_2$, and between venous PO_2 (PvO_2) and $\dot{M}VO_2$. Linear regression analysis was also applied to find the relationships between log RADO vs $\dot{M}VO_2$, log RADO vs PvO_2, and CF vs log $[ADO]_v$. We then tested for whether the two lines are identical, and for equality of their slopes (Neter and Wasserman, 1974). Differences between data for each age group were considered significant at $p<0.05$.

RESULTS

Effect of development on coronary flow (CF) and myocardial O_2 consumption ($\dot{M}VO_2$) during NE infusion

The dose-response relationship between CF (ml/min/g) and $\dot{M}VO_2$ (ul O_2/g/min) to NE infusion is presented in Figure 1. The maximum responses were obtained at NE concentrations of 1×10^{-6} to 5×10^{-6} M. Maximum coronary flow and maximum MVO_2 are significantly higher for 1 vs 18 month-old hearts. Table 1 summarizes the data for control, maximum, half maximum and the ED_{50} values of coronary flow and $\dot{M}VO_2$ for 1 and 18 month-old groups. All these values were significantly higher for 1 vs 18 month-old hearts.

Figure 2 shows the relationship between CF and $\dot{M}VO_2$ (Panel A) and $\dot{D}O_2$ and $\dot{M}VO_2$ (Panel B) of 1 vs 18 month-old hearts. The individual data points for CF and $\dot{D}O_2$ are plotted against $\dot{M}VO_2$. Control CF, $\dot{D}O_2$ and $\dot{M}VO_2$ were higher for 1 vs 18 month-old group ($p<0.05$). When $\dot{M}VO_2$ is raised by NE, $\dot{D}O_2$ and CF increase in proportion to $\dot{M}VO_2$ in both age groups. However, the intercept for $\dot{D}O_2$ and CF are significantly higher for 1 vs 18 month-old hearts. Given virtually identical slopes, we interpret this to mean that, for any $\dot{M}VO_2$, CF and $\dot{D}O_2$ are higher in the younger hearts. Figure 2B also shows that during metabolic stimulation, older hearts are closer to maximum O_2 extraction.

The relationship between $\dot{M}VO_2$ and pulmonary artery effluent $\dot{P}O_2$ (PvO_2) is shown in Figure 3. This figure shows that during NE administration, PvO_2 decreases with increasing $\dot{M}VO_2$ in both age groups. However, PvO_2 is significantly lower for 18 month vs 1 month-old hearts at all values of $\dot{M}VO_2$.

Effect of development on adenosine release (RADO):

The time-course of R_{ADO} during NE infusion for 1 and 18 month-old hearts is shown in Figure 4. R_{ADO} increased from control to maximal levels in 2 minutes, and declined to intermediate values in 4 to 6 minutes. The peak R_{ADO} values of the two age groups are plotted against $\dot{M}VO_2$ in Panel B of Figure 5. Panel A of this figure shows the mean steady-state values of R_{ADO} (6 - 12 minutes) for 1 and 18 month-old hearts as a function of their $\dot{M}VO_2$. Both peak and steady state R_{ADO} are significantly higher ($p<0.05$) for 18 vs 1 month-old hearts at a given value of $\dot{M}VO_2$. Steady-state and peak R_{ADO} values of the two age groups are plotted against PvO_2 in Figure 6, panels A and B, respectively. Both steady-state (panel A) and peak (panel B) R_{ADO} values are significantly higher ($p<0.05$) for 1 vs 18 month-old hearts at a given value of PvO_2.

Coronary flow (ml/min/g) is plotted as a function of peak venous adenosine concentration in Figure 7. Coronary flow is significantly higher ($p<0.05$) for 1 vs 18 month-old hearts at a given value of venous adenosine concentrations. These results suggest that the coronary bed of older hearts is less responsive to endogenous adenosine than the younger hearts.

Coronary flow in response to isoproterenol vs norepinephrine:

We tested the role of an age-related increase in alpha adrenergic receptor activity in limiting coronary flow and O_2 delivery to the 18 month-old hearts. Figure 8 shows the relationship between CF and $\dot{M}VO_2$ in twelve 18 month-old hearts. $\dot{M}VO_2$ was raised in six hearts by the administration of six different doses of isoproterenol (ISO). Six

different doses of NE were administered to the other six hearts in order to raise their $\dot{M}VO_2$. CF at a given $\dot{M}VO_2$ was not significantly different between ISO and NE groups. However, when PvO2 is plotted against $\dot{M}VO_2$, a difference in O_2 extraction is readily demonstrated between the two drugs. Figure 9 shows that P_vO_2, in the presence of NE is significantly lower than in response to ISO at higher levels of $\dot{M}VO_2$.

Table 1: Effect of development on coronary flow (ml/min/g) and $\dot{M}VO_2$ ($ul/O_2/g/min$) in response to norepinephrine:

	AGE			
	1 Month		18 Month	
	Coronary Flow	$\dot{M}VO2$	Coronary flow	MVO2
Control	4.7 ± 0.2*	61 ± 6*	2.7 ± 0.1	39 ± 3
Maximum	12.1 ± 0.9*	209 ± 18*	6.7 ± 0.2	114 ± 4
Half Maximum	8.4 ± 0.3*	132.2 ± 6*	4.8 ± 0.1	77 ± 3
ED_{50} of NE(M)	$5.9 \times 10^{-8} \pm 7.8 \times 10^{-9}$	$5.78 \times 10^{-8} \pm 5.7 \times 10^{-9}$*	$1.25 \times 10^{-7} \pm 2.9 \times 10^{-8}$	$1.3 \times 0^{-7} \pm 2.8 \times 10^{-8}$

Values are presented as mean ± SEM (n=6)

* $p<0.05$ vs 18 month-old group

Figure 1: The steady-state coronary flow and myocardial O_2 consumption (MVO_2) responses to various perfusate concentrations of norepinephrine. Values are presented as means $\pm$ SEM (n=6). When the SEM bars are not shown, they are smaller than the size of the symbols. * $P<0.05$ vs respective coronary flow and $\dot{M}VO_2$ values.

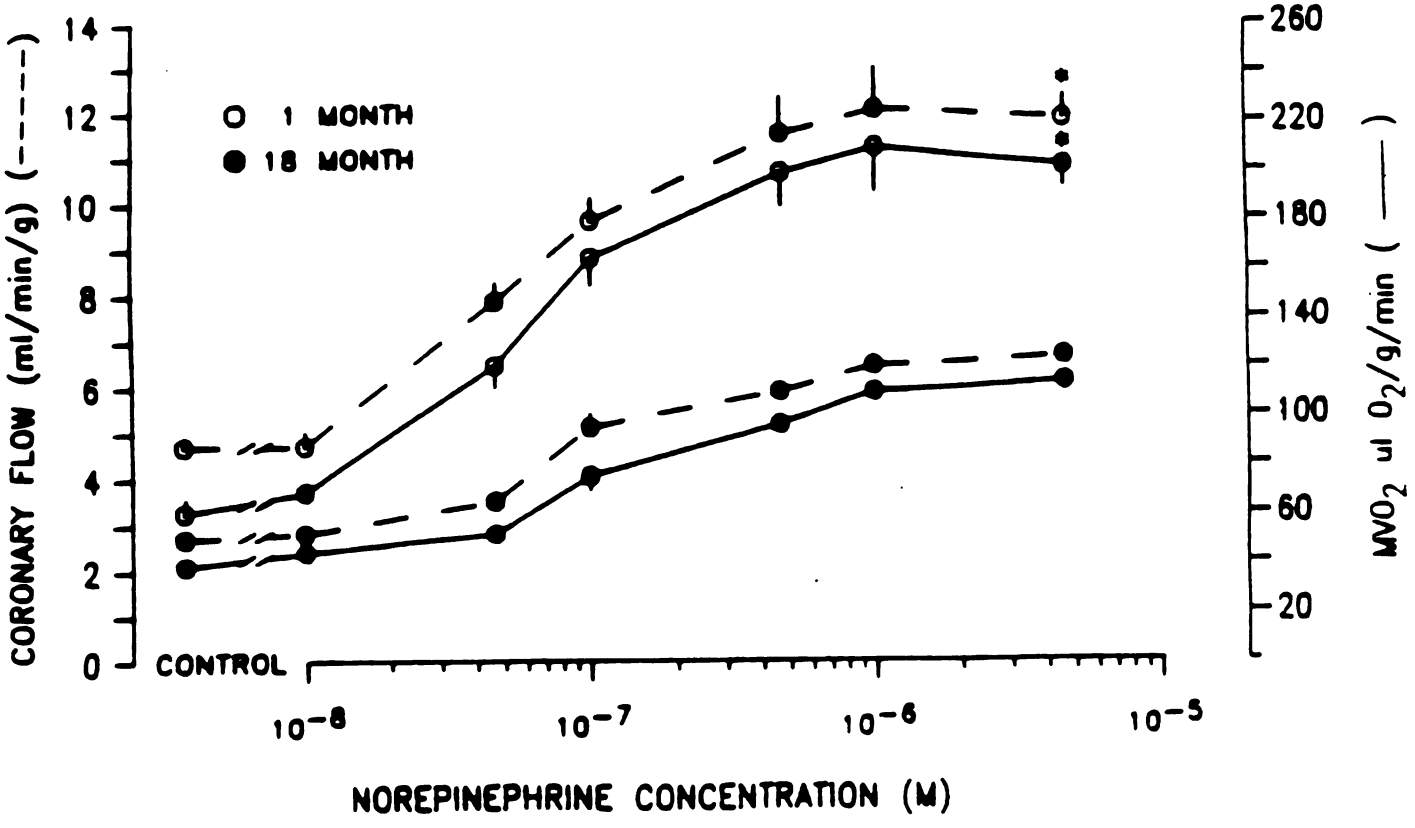
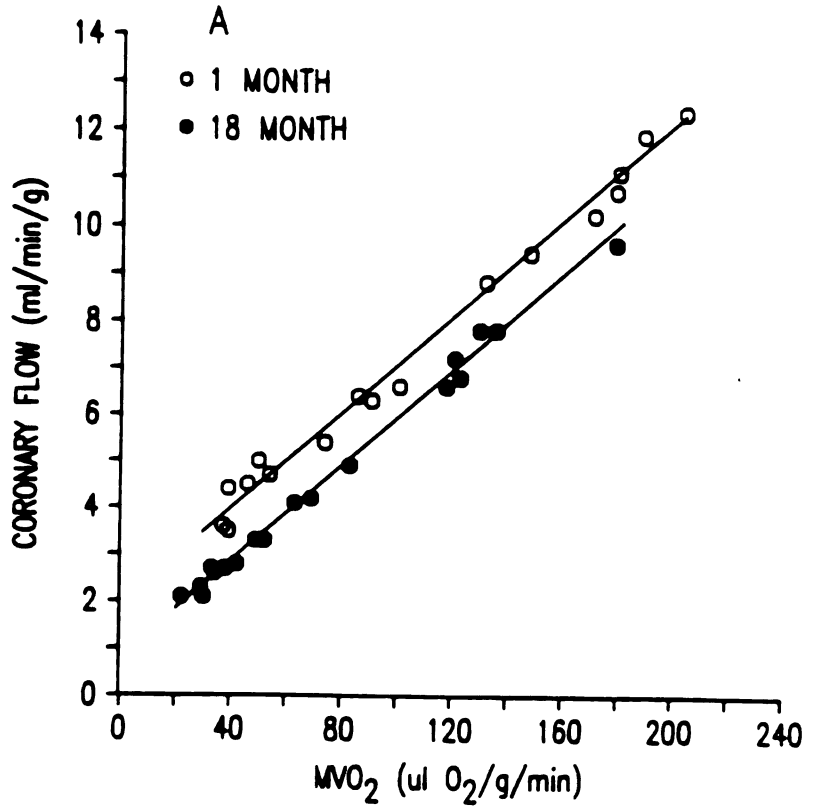

CORONARY FLOW (ml/min/g) (-----)
MVO2 ul O2/g/min (——)
O 1 MONTH
● 18 MONTH
CONTROL
NOREPINEPHRINE CONCENTRATION (M)
0
2
4
6
8
10
12
14
20
60
100
140
180
220
260
10-8
10-7
10-6
10-5

Figure 2 A: Relationship between myocardial O_2 consumption ($\dot{M}VO_2$) and coronary flow (CF) in 1 and 18 month-old guinea pig hearts. The equations which describe these relationships are: $CF = 1.96 + 0.05\ \dot{M}VO_2$ ($r = 0.995$) for 1 month and $CF = 0.82 + 0.05\ \dot{M}VO_2$ ($r = 0.996$) for 18 month-old group. $\dot{M}VO_2$ was raised by infusion of $5x10^{-8}$ and $5x10^{-6}$ (M) ME. For a given $\dot{M}VO_2$, CF is significantly less for 18 vs 1 month-old hearts.

B: Relationship between O_2 delivery ($\dot{D}O_2$) and $\dot{M}VO_2$ for 1 month-old group is described as $\dot{D}O_2 = 37.199 + 0.938\ \dot{M}VO_2$ ($r = 0.955$) and for the 18 month-old group is: $\dot{D}O_2 = 14.265 + 0.960\ \dot{M}VO_2$ ($r = 0.997$). The dashed line represents the theoretical limits of the plot, i.e., the locus of points associated with $PVO_2 = 0$ mmHg. $\dot{M}VO_2$ was raised same as in Panel A. At a given $\dot{M}VO_2$, CF and $\dot{D}O_2$ are significantly lower for 18 vs 1 month-old hearts.


A
1 MONTH
18 MONTH
CORONARY FLOW (ml/min/g)
MVO2 (ul O2/g/min)
0
2
4
6
8
10
12
14
0
40
80
120
160
200
240

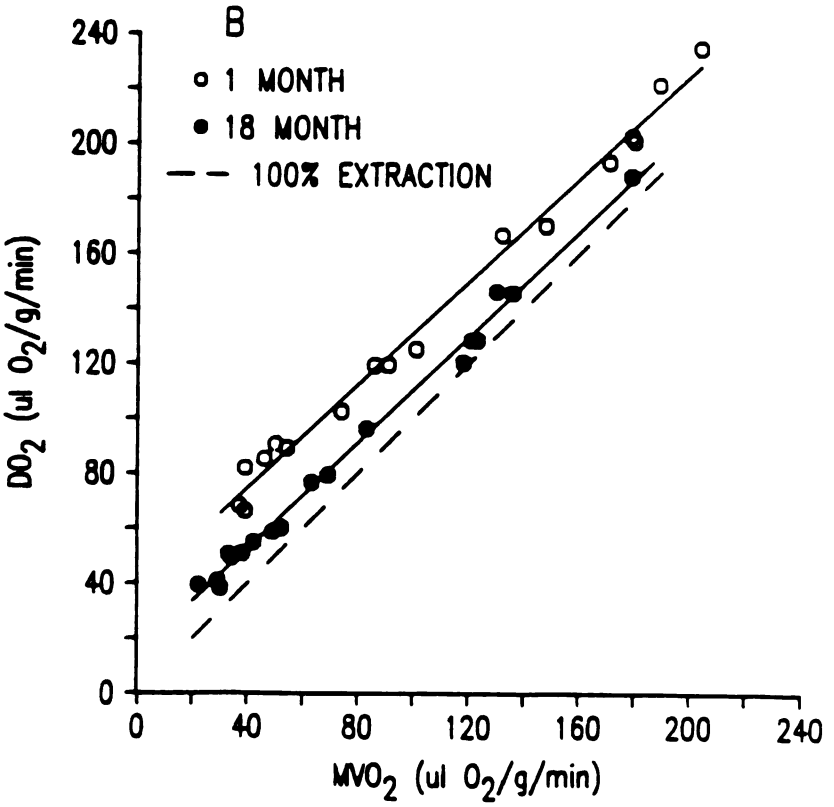

B
1 MONTH
18 MONTH
100% EXTRACTION
DO2 (ul O2/g/min)
MVO2 (ul O2/g/min)
0
40
80
120
160
200
240

Figure 3: Pulmonary artery effluent PO_2 (PvO_2) is plotted as a function of myocardial O_2 consumption ($\dot{M}VO_2$). MVO_2 was raised in a similar fashion as in Figure 2. PvO_2 is significantly lower ($p<0.05$) for 18 vs 1 month-old hearts at a given $\dot{M}VO_2$. This indicates higher O_2 extraction and lower tissue PO_2 for the 18 month-old hearts. The relationship between PvO_2 and $\dot{M}VO_2$ can be described in the following equations: $PvO_2 = 14551.8 / (12.0593 + \dot{M}VO_2)$ and $PvO_2 = 6158.99 / (2.10373 + \dot{M}VO_2)$ and $PvO_2 = 6158.99 / (2.10373 + \dot{M}VO_2)$ for 1 and 18 month old groups, respectively.

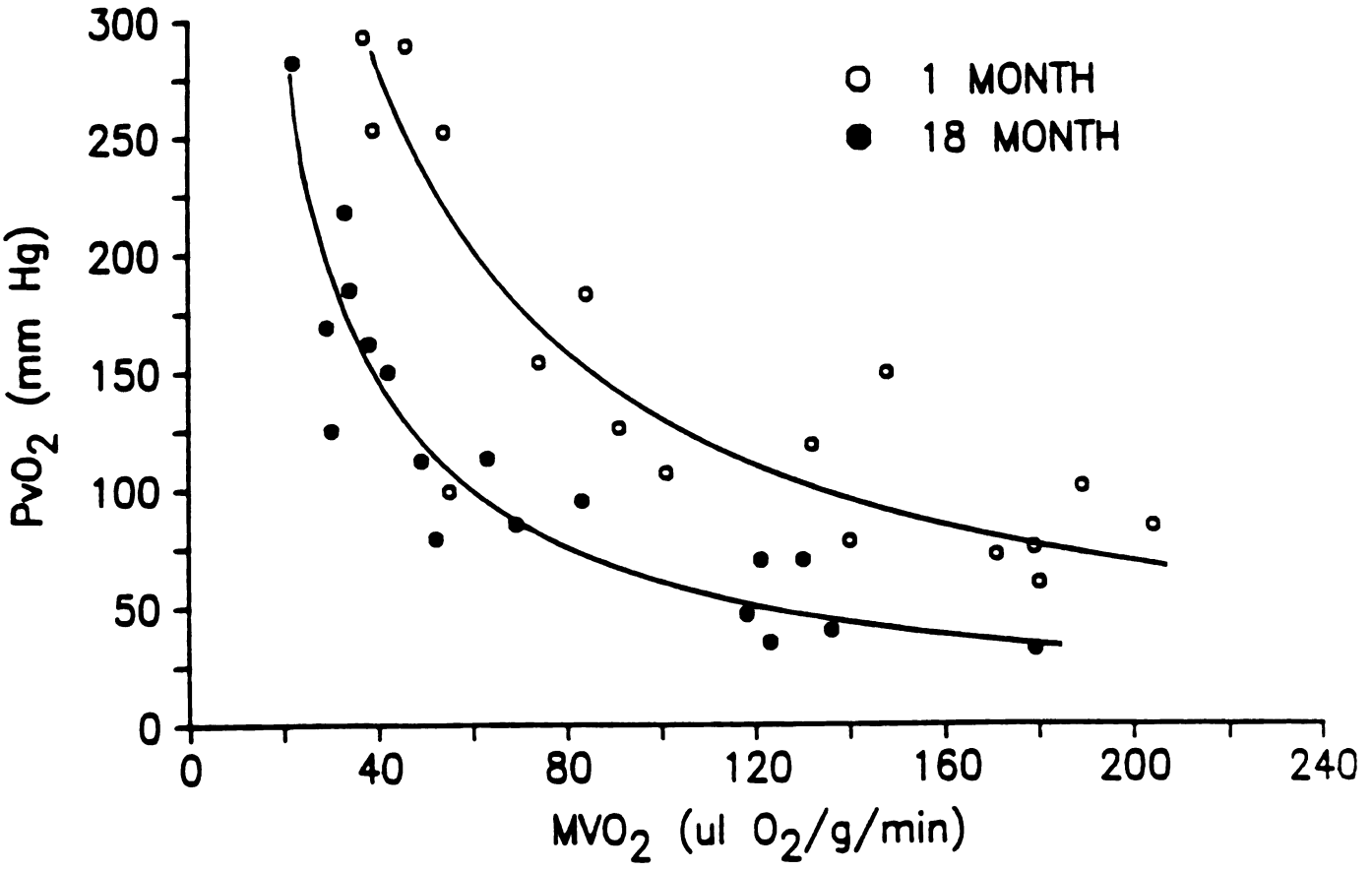

300
250
200
150
100
50
0
PvO_2 (mm Hg)
0
40
80
120
160
200
240
MVO_2 (ul O_2/g/min)
1 MONTH
18 MONTH

Figure 4: Adenosine release (R_{ADO}) is plotted as a function of time in 1 month and 18 month-old guinea pig hearts. During NE infusion (5×10^{-6} M). R_{ADO} increases from control to maximal levels within 2 minutes and declines toward steady-state levels within 4 to 6 minutes.

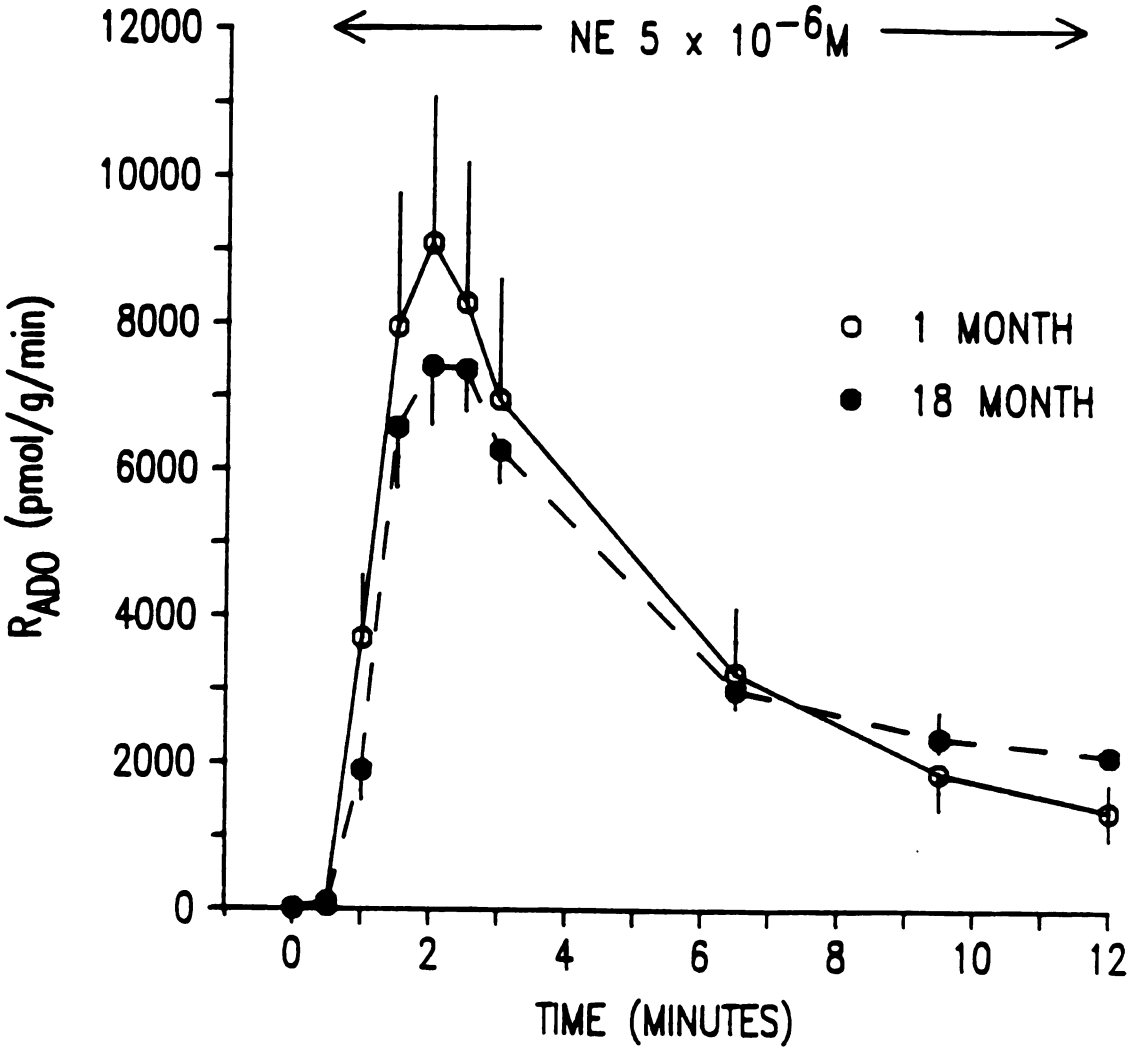
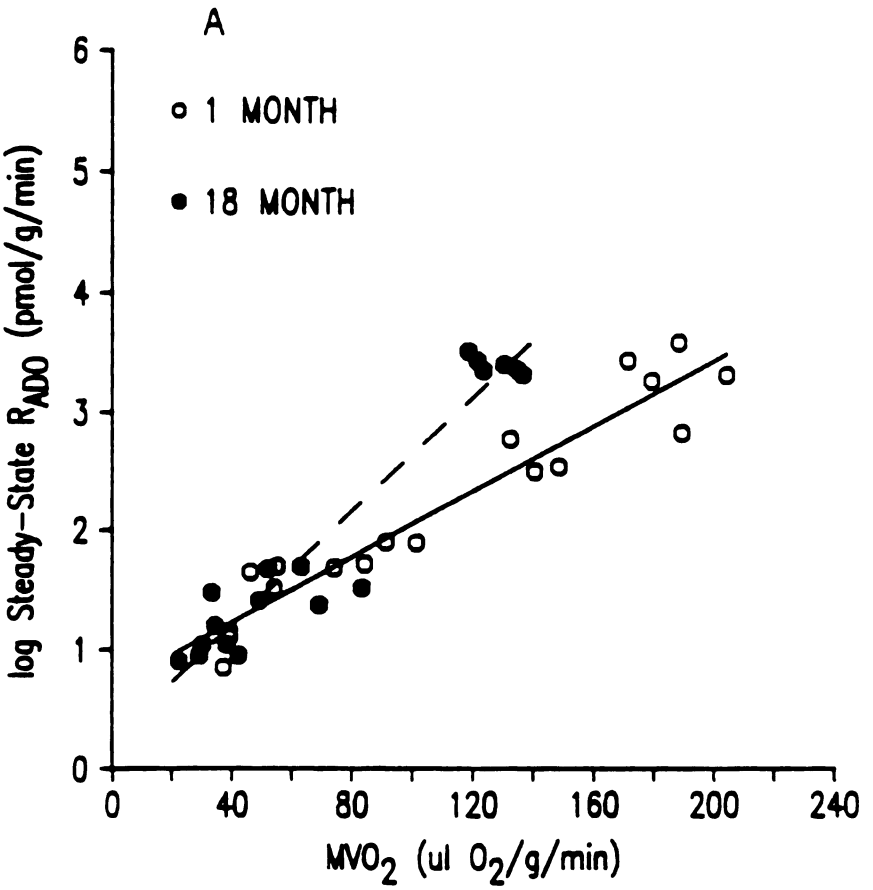

NE 5 x 10⁻⁶M
1 MONTH
18 MONTH
R_ADO (pmol/g/min)
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12
TIME (MINUTES)

Figure 5: A. The log steady-state adenosine release (RADO) is plotted as a function of myocardial O_2 consumption (MVO_2). This relationship is described by the following functions: log RADO = 0.68 + 0.014 MVO_2 (r=0.959) and log RADO = 0.416 + 0.021 MVO_2 (r=0.934), for 1 and 18 month-old hearts, respectively.

B. The relationship between log peak RADO and $\dot{M}VO_2$ is described in the following equations: log RADO = 0.683 + 0.018 $\dot{M}VO_2$ (r=0.953) and log RADO = 0.414 + 0.024 $\dot{M}VO_2$ (R=0.930) for 1 and 18 month-old hearts, respectively. $\dot{M}VO_2$ was raised by two doses of NE ($5x10^{-8}$ and $5x10^{-6}$ M). Since the two lines in each panel are statistically different ($p<0.05$), and virtually with similar slopes, this indicates that the differences between the lines is in the intercepts, ie for a given MVO_2, RADO is significantly greater for 18 vs 1 month-old hearts.


A
1 MONTH
18 MONTH
log Steady-State R_{ADO} (pmol/g/min)
MVO_2 (ul O_2/g/min)
0
1
2
3
4
5
6
0
40
80
120
160
200
240

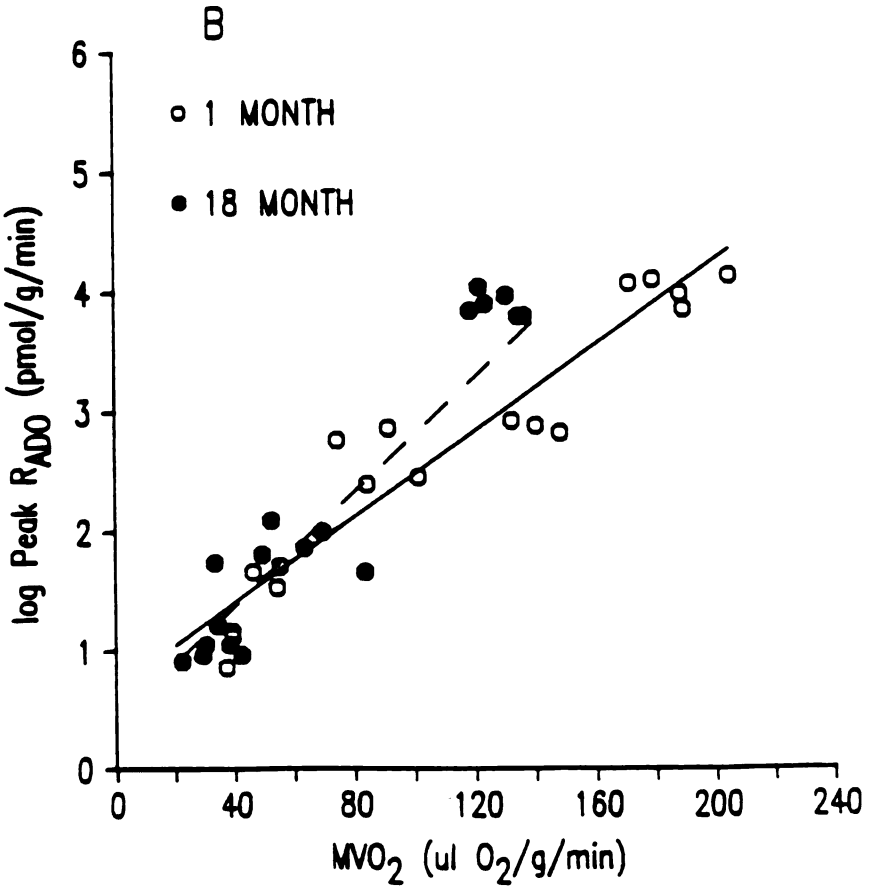
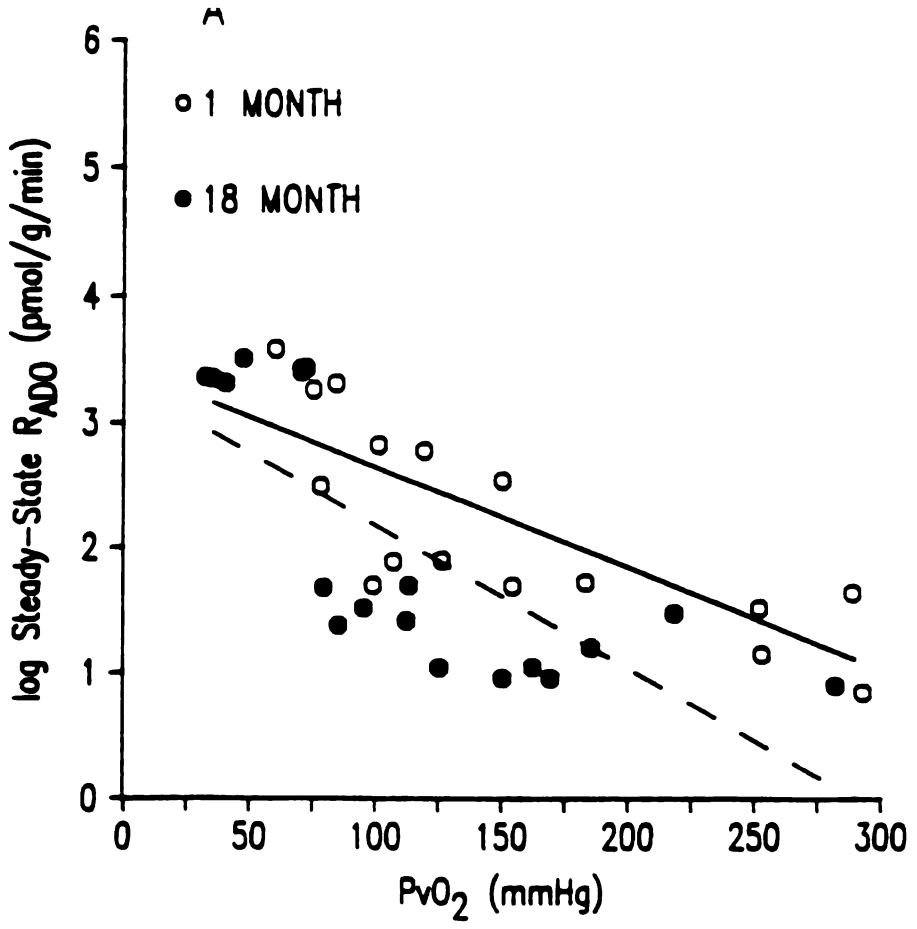

B
1 MONTH
18 MONTH
log Peak R_{ADO} (pmol/g/min)
MVO_2 (ul O_2/g/min)
0
1
2
3
4
5
6
0
40
80
120
160
200
240

Figure 6: This figure illustrates the steady-state (Panel A) and peak (Panel B) log adenosine release (RADO) plotted as a function of pulmonary artery effluent PO_2 (PvO_2) in 1 and 18 month-old hearts. PvO_2 was changed in response to NE infusion ($5x10^{-8}$ and $5x10^{-6}$ M). The relationship shown in Panel A can be described in the following equations: log RADO = 3.437 - 0.008 PvO_2 (r=-0.823) and log RADO = 3.340 - 0.012 PvO_2 (r = -0.770) for 1 and 18 month-old hearts, respectively.

The relationships shown in Panel B are described as follows: RADO = 4.3 - 0.011 PvO_2 (r = -0.854) and RADO = 3.904 - 0.014 PvO_2 (r= -0.791) for 1 and 18 month-old hearts, respectively.

The two regression lines of each panel are significantly different ($p<0.05$) from each other. Because their slopes are similar, thus the difference between each two lines is in their Y intercept. This indicates that RADO is significantly lower for 18 vs 1 month-old heart at the same PvO_2.


A
1 MONTH
18 MONTH
log Steady-State R_{ADO} (pmol/g/min)
PvO_2 (mmHg)
0
1
2
3
4
5
6
0
50
100
150
200
250
300

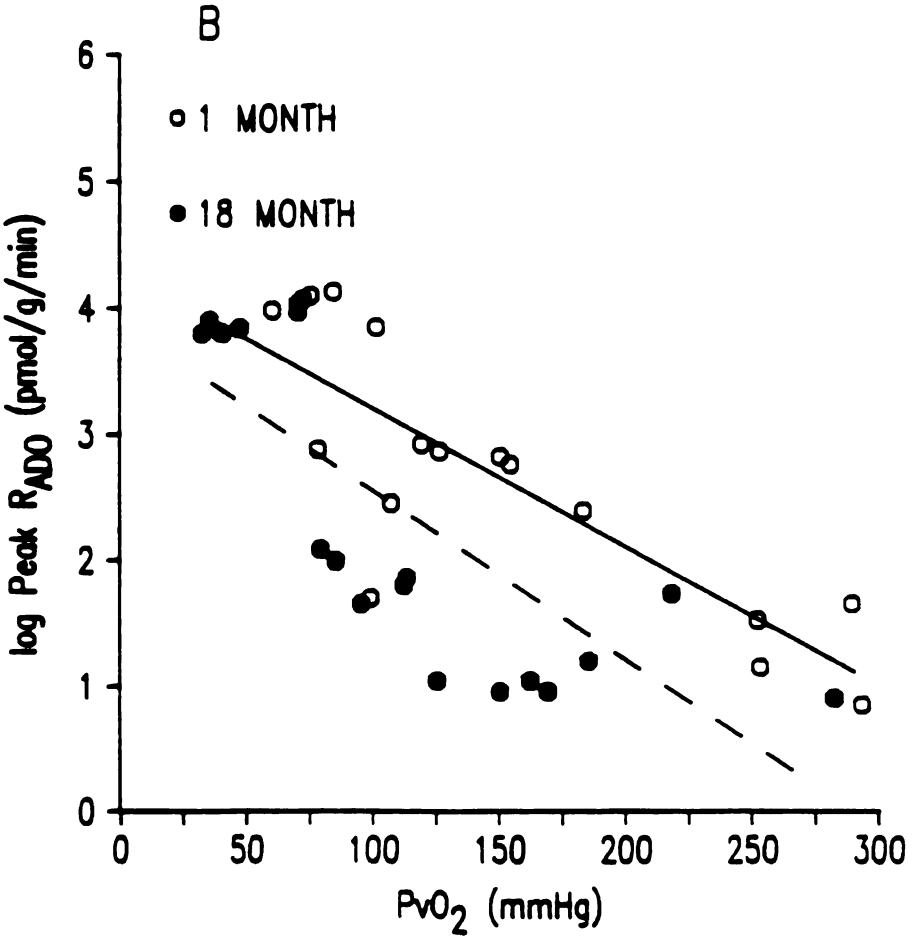

B
1 MONTH
18 MONTH
log Peak R_{ADO} (pmol/g/min)
PvO_2 (mmHg)
0
1
2
3
4
5
6
0
50
100
150
200
250
300

Figure 7: Shown in this figure is the relationship between coronary flow (CF) and peak venous adenosine concentration. Venous ado concentration was raised by increasing myocardial metabolism with two doses of NE ($5x10^{-8}$M and $5x10^{-6}$M). Coronary flow is higher for 1 vs 18 month-old hearts at a given venous adenosine concentration. The relationship between venous adenosine and CF is described in the following function: CF = 2.359 + 2.754 log X (r = 0.892) for the 1 month group, and CF = 1.144 + 2.084 log x (r = 0.925) for the 18 month-old hearts.

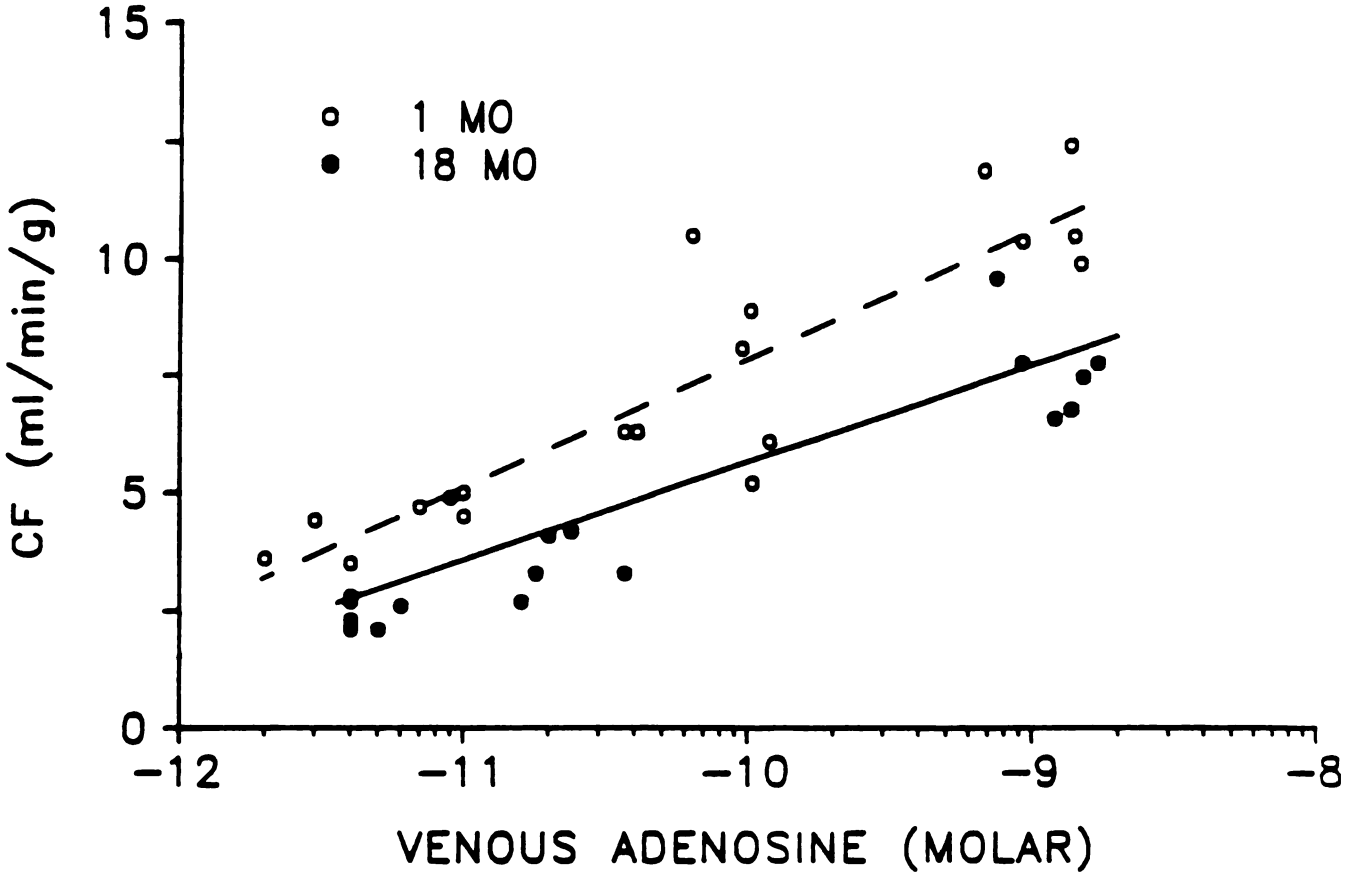

15
10
5
0
CF (ml/min/g)
1 MO
18 MO
−12
−11
−10
−9
−8
VENOUS ADENOSINE (MOLAR)

Figure 8: The effects of norepinephrine (solid line) vs isoproterenol (dashed line) on the relationships between coronary flow (CF) and myocardial O_2 consumption ($\dot{M}VO_2$) in the hearts of 18 month-old guinea pigs. The functions that describe these relationships are: CF = 0.624 + 0.055 MVO_2 (r = 0.994) for the isoproterenol group and CF = 0.973 + 0.052 MVO_2 (r = 0.980) for the NE group. $\dot{M}VO_2$ is raised by 6 doses of NE ranging from $5x10^{-8}$ to $1x10^{-5}$M or by 6 doses of ISO ranging from $5x10^{-9}$M to $5x10^{-6}$ M. At a given MVO_2, CF is similar between the isoproterenol and NE-treated hearts.

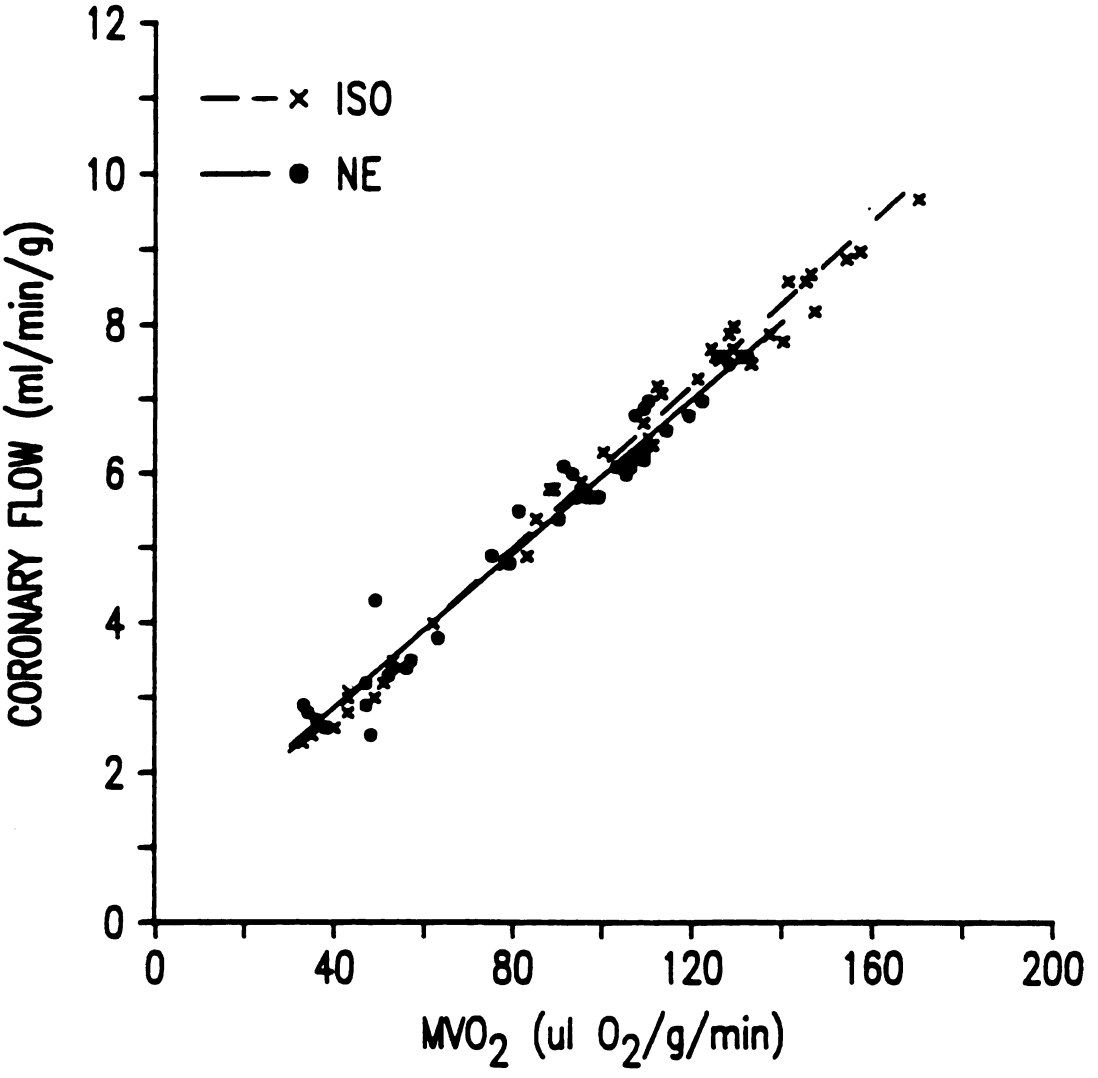
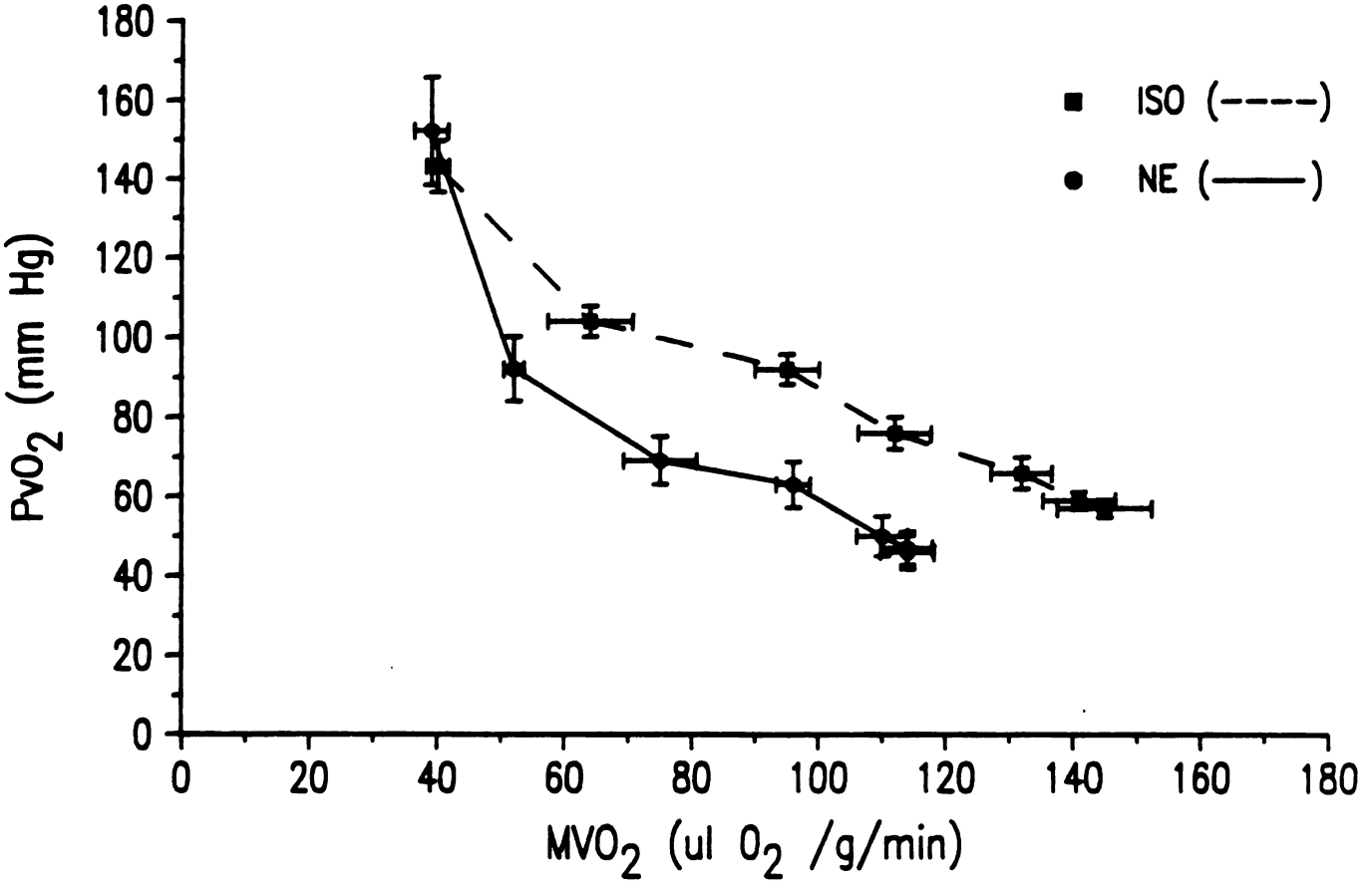

12
10
8
6
4
2
0
ISO
NE
CORONARY FLOW (ml/min/g)
0
40
80
120
160
200
MVO2 (ul O2/g/min)

Figure 9: The effects of isoproterenol (ISO) and norepinephrine (NE) on the relationship between pulmonary artery effluent PO_2 (PvO_2) and myocardial O_2 consumption (MVO_2) in the 18 month-old hearts. MVO_2 is raised by 6 doses of ISO ranging from 5×10^{-9} M to 5×10^{-6} M or by 6 doses of NE ranging from 5×10^{-8} to 1×10^{-5} M. At a given MVO_2, PvO_2 is significantly higher for ISO-treated hearts than for NE-treated hearts.


180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
120
140
160
180
PvO2 (mm Hg)
MVO2 (ul O2 /g/min)
ISO (----)
NE (——)

DISCUSSION

The major findings of this study can be summarized as follows:

1) During metabolic stimulation with NE, coronary flow and oxygen delivery in 18 month hearts are reduced when compared to 1 month-old hearts with the same MVO_2 (Figure 2).
2) Oxygen extraction and release of adenosine are increased in 18 vs 1 month-old hearts with the same MVO_2 (Figures 3 and 5).
3) Increased alpha-adrenergic activity in the 18 month-old heart is not the major factor responsible for the lower coronary flow during NE infusion (Figure 8)

We have previously demonstrated that the developmental process is associated with a reduced coronary vasodilator reserve in response to exogenous adenosine and sodium nitroprusside infusions and to transient coronary occlusions (Toma et al., 1985). In the current study, we tested the hypothesis that this decrement in vasodilator reserve limits O_2 supply and coronary flow to the mature heart when its demands for O_2 consumption are increased. The results depicted in Figure 2 show that when $\dot{M}VO_2$ is increased by NE, coronary flow and O_2 supply are significantly decreased for 18 vs 1 month-old hearts at all values of $\dot{M}VO_2$. It follows that 18 month-old hearts extract more of the delivered O_2 than do 1 month-old (Figures 2 and 3). Thus, it appears that cardiac development is associated not only with decreases in vascular density (Tomanek et al., 1982) and pharmacologic vasodilator reserve (Toma et al., 1985), but also, and perhaps more importantly with a decreased metabolic or functional reserve.

In our previous report (Toma et al., 1985), the maximum pharmacologic reserve (induced by adenosine) was 18.5 $\pm$ 1.4 and 10.6 $\pm$

0.8 ml/min/g for 1 and 18 month-old hearts, respectively. In contrast, the maximum metabolic vasodilator reserve reported here (obtained by NE) is 11.9 $\pm$ 0.4 and 7.6 $\pm$ 0.4 ml/min/g for 1 and 18 month age groups, respectively. This means that the "pharmacologic" reserve exceeds the metabolic vasodilator reserve by 40% and 28% for 1 and 18 month-old hearts, respectively. Several studies have demonstrated that the magnitude of the coronary reserve depends on the applied stimulus (i.e. pharmacologic vs physiologic) in producing maximum coronary blood flow (Hoffman, 1984). Bookstein et al. (1977), and Warltier et al. (1981), have shown, in dogs, that the maximum coronary blood flow in response to infusion of various vasodilators like adenosine, ATP, chromonar or papaverine is higher than the peak reactive hyperemic response following a 90 second occlusion period. Furthermore, Barnard et al. (1977) reported that the large increase in coronary flow produced by near-maximum exercise in dogs could be increased another 35% by dipyridamole. Thus it appears that there is a marked difference between "pharmacologic" and "physiologic" coronary reserves. Furthermore, when the age of the experimental animal is considered, the 1 month-old heart has a larger unused coronary reserve than has a mature 18 month-old heart. Why this large pharmacologic reserve was not used in our experiments is unknown. It is concluded from these results that the developmental process decreases the "pharmacologic" as well as the "physiologic" vasodilator capacity of the growing myocardium. In addition, it seems that, at least by this stimulus, hearts from young animals (1 month-old) have a greater unusable coronary vasodilator reserve than the mature (18 month-old) hearts.

Adenosine has been postulated to mediate the increase in coronary flow during increased myocardial metabolism (Berne, 1980). Despite controversy about the quantitative role of adenosine in mediating metabolic vasodilation, it is one of the most well studied mediators (Belloni, 1979; Berne et al, 1983; Feigl, 1983; Sparks et al, 1984). One of the physiological signals for adenosine formation is a decrease in the O_2 supply-to-demand ratio (Berne, 1964; Bardenheuer and Schrader, 1983). That is, whenever myocardial metabolism is raised relative to O_2 delivery, adenosine release is stimulated. Our finding that coronary flow and O_2 delivery, relative to $\dot{M}VO_2$, decrease with maturation, suggests that the O_2 supply/demand ratio is lower in older hearts. Based on the supply/demand hypothesis we predicted that for a given value of $\dot{M}VO_2$ adenosine release would be higher in the 18 than 1 month-old hearts. The results shown in Figure 4 indicate that, in fact, our hypothesis is true. Adenosine release is higher for the 18 vs 1 month-old group, at given values of MVO_2. Increased adenosine release by 18 month-old hearts is apparently because they have a lower coronary responsiveness to exogenous adenosine (Toma et al., 1985).

To test whether the increased release of adenosine in the 18 month-old hearts is related to a lower tissue PO_2 (Figure 3), adenosine release was plotted as a function of PvO_2 in Figure 6. The results depicted in Figure 6 show that R_{ADO} is higher for 1 than 18 month-old groups at a given PvO_2. These data suggest that even though the 18 month-old hearts have a higher R_{ADO} at a given MVO_2, this increment is not related to a lower tissue PO_2, provided that PvO_2 reflects tissue PO_2. Other physiological signals may contribute to the augmented R_{ADO} in the 18 month-old hearts. Accumulating evidence suggests variable

sources and signals for adenosine formation (Sparks and Bardenheuer, 1985). A possible source may be from AMP formed by the action of phosphodiesterase on cAMP formed during catecholamine stimulation. However, this cannot explain the augmented R_{ADO} at a given MVO_2 in the older hearts (Figure 5), because adenyl cyclase activity and cAMP production are decreased in older rodent hearts (Narayanan and Derby, 1982). On the other hand, this may explain the apparent discrepancy between the results of Figures 5 and 6. The greater RADO at a given P_vO_2 (Figure 6) could be the result of higher cAMP levels, which in turn, lead to the increased adenosine formation in the one month-old hearts. Nevertheless, the physiological signal(s) which trigger(s) the higher R_{ADO} at a given $\dot{M}VO_2$ in the older hearts remain(s) unknown. It is beyond the scope of this study to explore the possible source(s) and mechanisms(s) of the augmented R_{ADO}.

The greater increase in myocardial adenosine production may have other effects on the 18 month-old hearts. Adenosine has been known to interfere with many effects of the beta adrenergic activities in the heart. It inhibits NE release from sympathetic nerve endings (Verhaeghe and Vanhoutte, 1977), reduces atrioventricular node conduction velocity (Belardinelli et al., 1983),and exerts negative inotropic (Dobson and Fenton, 1983) and negative chronotropic effects (Belardinelli et al., 1983). Our results (Figure 1) and several studies on rodents and human hearts have shown an age-related decrease response to beta-adrenergic agonist during development (Conway, 1970; Williams and Thompson, 1973; Vestal et al., 1979; Buhler et al., 1980; Narayanan and Derby, 1982). Part of the decreased response is a decreased receptor activation. We propose that the augmented release of adenosine in the

18 month-old heart is another factor responsible for decreased beta-adrenergic response of the older heart.

In the isolated guinea pig heart, maturation results in a lower O_2 delivery for a given MVO_2. This may be the result of a reduced responsiveness to adenosine and/or decreased vascular cross-sectional area. Older hearts release more adenosine at a given MVO_2, but this is not a result of a lower PvO_2. Nonetheless, the endogenous adenosine, despite its augmentation, does not increase coronary flow to levels seen in younger hearts (Figure 7).

The relaxing effect of isoproterenol (ISO) on isolated rat aortic rings has been shown to be decreased dramatically between 1 and 3 months, and completely abolished by 6 months of age (Fleisch et al., 1970; Ericsson and Lundholm, 1975). A decline in the response of isolated coronary strips from young and old horses to ISO was shown by Siro-Brigiani and Chieppa (1965). However, the age differences in vasodilation, in response to NE reported here, are not likely to be solely due to differences in the beta-adrenoceptor-mediated mechanism, since we have shown differences in responses to other vasodilators (Toma et al., 1985). On the other hand, Buhler et al. (1980) and Elliott et al. (1982) have indicated that increasing age may be associated with enhanced alpha-adrenoceptor-mediated vasoconstriction of the peripheral vessels. A possible explanation for the limitation of myocardial perfusion and O_2 delivery to the 18 month-old group is enhanced alpha adrenergic receptor response of the coronary vessels. Since ISO has no adrenergic alpha receptor activity, we compared the effects of ISO and NE on the relationship between MVO_2 and CF in twelve 18 month-old hearts (Figure 8). As demonstrated in Figure 8, at a given MVO_2, there is no

significant difference in the CF of ISO and NE treated groups. This result argues against the hypothesis that O_2 supply of the 18 month-old hearts is limited by alpha adrenergic receptor activity. However, when PvO_2 is plotted against MVO_2, the NE-treated hearts do have a higher O_2 extraction than the ISO-treated group at high levels of MVO_2 (Figure 9). This effect of coronary alpha-adrenergic activity on O_2 extraction has been previously shown by other investigators in the blood-perfused dog hearts (Mohrman and Feigl, 1978; Rooke and Sparks, 1980).

An alternative possible explanation for the differences in CF between the two age groups in response to NE (Figures 1 and 2 A), which has not been tested in this study, is a decrease in the vasodilator activity of the endothelial $alpha_2$ adrenergic receptors in the 18 month-old hearts. Cocks and Angus (1983) have shown that NE causes the release of a relaxing factor from endothelial $alpha_2$ receptors of coronary artery strips. They further suggested that the net effect of NE is determined by the algebraic sum of the contractile and relaxing factors. Recent evidence has demonstrated a decreased $alpha_2$ population in aortic, mesenteric and renal arteries of 4 to 8 vs 16 week-old rabbits (Tayo et al., 1985).

In summary, we have demonstrated that the age-related decrease in the pharmacologic coronary vasodilator reserve observed in our earlier study (Toma et al., 1985) is associated with a decreased metabolic reserve as well. This is evidenced by the reduced coronary flow and O_2 delivery to the 18 month vs 1 month-old hearts at a given increase in $\dot{M}VO_2$ caused by infused NE. The 18 month-old hearts release more adenosine and extract more O_2 for a given increase in O_2 consumption. Furthermore, the decreased dilator response to NE does not appear to be

due to enhanced alpha-adrenergic vasoconstrictor activity. Instead we propose that it is due to failure of microvasculature to keep pace with myocardial growth, resulting in a decreased vascular density and vascular cross-sectional area. In conclusion, the age of an animal is an important factor in determining the magnitude of metabolic coronary reserve and release of adenosine.

CHAPTER V GENERAL DISCUSSION

V.1. Summary of the major findings:

The major findings reported in this thesis can be summarized as follows:

1) The age-related decline in capillary density and capillary surface area of the mammalian heart reported by others (Wearn, 1941; Rakussan et al., 1965; Tomanek et al., 1982) is associated with a similar age-related decrease in the coronary vasodilator reserve (CVDR) of the developing guinea pig heart. The maximum coronary flow per gram heart weight in response to adenosine (ado), sodium nitroprusside (SNP), and transient coronary occlusion, is highest in the neonate and declines with heart growth until sexual maturity, i.e. 2 months, of age (Figures III-1 and -2, Table III-3). It remains relatively constant during adult life.

2) Total coronary flow (an index of total vascular cross-sectional area of the heart) increased with development. However, it does not increase in proportion to heart weight until sexual maturity (Figure III-3 and Table III-1).

3) Despite decreased capillary density, and decreased CVDR, coronary autoregulatory capacity is not altered (Figures III-4 and -5).

4) CVDR is decreased during metabolic stimulation with NE (Figure IV-1). In this situation, coronary flow and oxygen delivery for the 18-month-old hearts are significantly reduced when compared to the 1 month-old hearts, with the same $\dot{M}VO_2$ (Figure IV-2A and B).

5) The lower coronary flow and O_2 delivery of the older hearts are compensated by increased oxygen extraction (Figures IV-2A and -3).

6) During metabolic stimulation with NE, the older hearts release more adenosine than the younger ones (Figure IV-5).

7) Increased alpha-adrenergic receptor activity in the 18 month-old hearts is not the major factor responsible for the decreased coronary flow and oxygen delivery during NE infusion (Figure IV-8).

V.2. Critique of methods

The isolated, non-working, crystalloid perfused heart preparation used in these studies has been well characterized in our laboratory (DeWitt et al., 1983; Wangler et al., 1984) and other laboratories (Bunger et al., 1975; Schrader et al., 1977). There are several advantages to using this type of preparation for the study of the effects of myocardial growth on coronary vasodilation and adenosine release. An isolated preparation allows us to draw conclusions about potential vasodilator capacity of the coronary bed, unobscured by the effects of nerves or circulating vasoactive substances. Furthermore, crystalloid, rather than blood perfusion, simplifies the measurements of myocardial adenosine production. This is because the perfusate is of known composition and does not degrade adenosine. A limitation of this preparation is that we do not know to what extent our results can be applied to blood-perfused hearts.

The purpose of these studies was to examine maximum coronary conductance and adenosine release during development. For these purposes, the Langendorff preparation does exhibit many pertinent features of an intact, blood-perfused heart. Spontaneous heart rate, coronary flow, and oxygen consumption are stable, and reactive hyperemic responses are reproducible for up to 3 to 4 hours; this exceeds the time

of experimentation. This preparation exhibits autoregulation over a wide range of perfusion pressures (Bunger et al., 1975; Schrader et al., 1977). It has a basal coronary flow, a maximum flow and reactive hyperemic response which are similar to blood-perfused rodent hearts (Wangler et al., 1982; Peter et al., 1984). These observations indicate that oxygen delivery to these heart is adequate for their oxygen consumption.

The guinea pig has been used previously for the study of age-related changes in the cardiovascular system. Changes in myocardial capillary density (Petren and Sylven, 1937), cardiac performance and electrical activity (Rumberger and Timmermann, 1976) and aortic smooth muscle relaxation (Fleisch et al., 1970) have been reported. It is possible that functional changes similar to those observed in guinea pigs occur in the human heart because the same reduction in capillary density has been reported for human beings and guinea pigs.

The average life span of a guinea pig is 6 to 7 years; and the average age of sexual maturity is 55 to 70 days (Rogers, 1950). The youngest age used in this study is 1 week at which age guinea pigs are rapidly growing but still dependent on their mothers for milk. The 1 month-old guinea pig is independent of mothers' milk and still rapidly growing. The 2 month-old guinea pig has reached sexual maturity but is still growing. The 12 and 18 month-old animals are no longer growing but have not entered senescence (above 4 years of age). The findings reported in this thesis are considered to be changes associated with development, not senescence.

V-3. Factors influencing coronary vasodilator reserve.

As mentioned earlier in chapters III and IV, the decreased CVDR with age is probably because of the failure of the coronary vascular bed to keep pace with myocardial growth. This conclusion was drawn based on the followings:

1) The decreased CVDR could not be the result of down regulation of a specific type of receptor, since maximum coronary flow in response to ado and SNP, and during reactive and functional hyperemia are all reduced with increasing age.

2) Maximum coronary flow in adenosine arrested hearts is also age dependent. This suggests that the decline in CVDR is not due to an increased extravascular or wall compressive forces, which may increase with age (Yin et al, 1980).

3) Although vascular stiffness increases with age, this, however, does not account for the decreased CVDR since calculations of gains of the autoregulatory curves demonstrated comparable degrees of passive elasticity in all age groups at high perfusion pressures.

The decline in CVDR is probably the result of one and/or a combination of the following factors:

1) It is possible that decreased CVDR results from impaired smooth muscle relaxation. Although impaired relaxation resulting from a decreased Ca^{++} reuptake ability has been demonstrated in cardiac muscle, such a possibility has not been tested on vascular smooth muscles.

2) Currently we do not have solid evidence to determine whether the increased vascular resistance is the result of decreased capillary density or a decrease in another microvascular segment such as the

density of arterioles. However, our results lead us to speculate that the density of small resistance vessels may also be reduced during development, since maximum total coronary flow in response to ado was significantly higher than that in response to SNP in the 1 week and 1 and 2 month-old hearts but not in the 12 and 18 month old hearts (Figure III-3). See the discussion section of Chapter III for details.

3) Although neither of these possibilities was tested in our studies, we think that failure of the coronary bed to keep pace with myocardial growth and the resultant decrease in CD is the most likely reason behind the decline in CVDR. This is because the time-course for the decline in coronary vasodilator reserve, reported in this study, follows that of the decline in vascular density (Tomanek et al., 1982). In view of this, it is interesting to consider the factors involved in capillary proliferation of the mammalian heart.

Factors involved in capillary growth:

There are two basic groups of factors considered responsible for capillary proliferation in the heart, namely, chemical and mechanical factors (Hudlicka, 1982 and 1984).

1. Chemical factors:

Hypoxia is one of the chemicals influencing cardiac capillary proliferation which has been extensively studied. Hypoxia is proposed to stimulate capillary proliferation in several tissues. Ashton (1961) suggested that endothelial cells of retinal capillaries are in some way directly sensitive to oxygen, multiplying at low oxygen levels, resting at normal and dying at high oxygen concentrations. It has long been

known that highly oxidative (red) muscles, such as cardiac muscles, are capable of being continuously active, have a higher activity of oxidative enzymes, capillary density, blood flow and O_2 consumption than the white muscles (Hudlicka et al., 1973). A good correlation has been shown between the activity of certain oxidative enzymes, such as citrate synthase and succinate dehydrogenase, and capillary density on one hand, and between exposure to hypoxia and activity of these enzymes on the other hand (Hudlicka, 1982). Thus hypoxia and capillary density are indirectly correlated. Hudlicka (1984) hypothesized that highly oxidative muscle fibers are able to extract more oxygen from the blood and producing local regions of hypoxia. This in turn, stimulates the proliferation of endothelial cells and promotes capillary growth as hypothesized by Ashton (1961). If this is true for cardiac muscles, then one might expect that exposure to hypoxia should result in increased capillary density. However, the results of such studies are inconsistent. Long term exposure of rats to hypoxic environment resulted in hypertrophy and increased capillary density of the right ventricle but not the left ventricle (Turek et al., 1975). These authors suggested that low PO_2 per se cannot be the stimulus for capillary growth. Rather, capillary proliferation is more related to the increased work performance by the right ventricle to overcome the increased pulmonary vascular resistance induced by hypoxia and the subsequent increase in blood flow to the right ventricle. This conclusion is supported by the finding that polycythemia, induced by repeated blood transfusions, resulted with a similar increase in capillary density produced by high-altitude hypoxia (Miller et al., 1970). On the other hand, a recent study by Kayar and Banchero (1985)

provided evidence to support this hypothesis. These investigators showed that exposure of guinea pigs to hypobaric hypoxia resulted in comparable degrees of hypertrophy of both the right and left ventricles. In addition, this was associated in similar increases in capillary density and capillary-to-fiber ratio, and similar decrease in the diffusion distance, of both ventricles. These findings suggest that hypoxia may indeed stimulate capillary growth. In conclusion, it appears that the role of low PO_2 per se in promoting capillary proliferation is still controversial.

Several blood-born chemical agents are purported to cause capillary proliferation during certain physiologic and pathophysiologic processes such as wound healing and tumor growth in a variety of organs and tissue cultures. Examples on such stimulating factors are ADP and serotonin released from platelets (Saba and Mason, 1975), lysosomal enzymes released from polymorpholeukocytes (Saba et al., 1978), heparin and histamine released from mast cells (Azizkhan et al., 1980) and progesterone (Hudlicka, 1984); whereas estrogen, cortisone and adrenocorticotropic hormone diminished capillary proliferation (Hudlicka, 1984). The role of these chemical factors in promoting myocardial capillary proliferation has not been studied. Of interest, are the hormonal factors, especially sex hormones; since the results of this study (Chapter III) showed that the decline in CVDR occurs at the time of sexual maturity.

A recent study by Chilian et al (1985) provided convincing evidence that chronic thyroxin treatment results in an increase in capillary density and coronary vasodilator reserve of 3 and 7 month-old rats in comparison to their age-matched controls.

An angiogenic factor known as Tumor Angiogenesis Factor (TAF), a protein of 10^5 dalton, has been isolated from a variety of tumor cells obtained from different animal species (Folkman and Cotran, 1976). TAF has a mitogenic effect on capillary endothelial cells. It has been proposed that TAF promotes capillary proliferation and vascularization during tumor growth. It is unknown whether factors such as TAF are involved in normal tissue capillary growth.

2. Mechanical factors:

It is possible that increased coronary blood flow (CBF) resulting from exercise, chronic treatment with vasodilating agents and long-term bradycardial pacing could cause vascular proliferation. Thoma (1893) first proposed a role for increased blood flow in stimulating capillary growth based on his observations on chicken embryos. This was later confirmed by Clark (1918) using tadpole tails and rabbit ears. Whereas the former postulated that increased blood flow through the terminal vascular bed leads to its mechanical expansion and stimulates capillary growth, the latter author hypothesized that mechanical friction of endothelial lining stimulates capillary growth. On the other hand, Branemark (1965) argued that pulsatory movement of erythrocytes during the first phase of vascularization are of primary importance for capillary growth. Since addition of fresh serum enhanced the proliferation of cultured endothelial cells, Gospodarowicz et al. (1978) suggested that a combination of cell geometry (i.e. mechanical expansion) and some mitogenic factors may be responsible for capillary proliferation both in vitro and in vivo.

2.a. Role of exercise:

It is well known that hearts of more active animals of similar species have a higher capillary density (CD). For instance, hearts of hares have higher CD than rabbits, and similarly wild rats have higher CD compared to laboratory rats (Wachtlova et al., 1965 and 1967). Exercise is also associated with increased CBF. It might thus be expected that training would stimulate capillary growth and increase CD in the heart. Although the results of such studies are quantitatively controversial, there is a general agreement that several patterns of exercise lead to increase CD in adult normotensive rats (Tomanek, 1970; Bloor and Leon, 1970) as well as in adult spontaneously hypertensive rats (SHR) (Crisman et al., 1985). If increased blood flow mechanically stimulates capillary growth, increased CBF during exercise and in athletic animals (the hares and wild rats), might account for a higher CD.

2.b. Chronic pharmacologic vasodilation:

If increased CBF per se during exercise stimulates capillary growth, the chronic administration of a vasodilator to a resting heart would stimulate capillary proliferation and increase CD. In fact, this has been shown to be true. Tornling et al. (1978) showed a greater incorporation of ^{3}H-thymidine in heart capillaries of young rats chronically treated with dipyridamol. Furthermore, Tornling (1982) showed that 6 week treatment with 3 mg/kg dipyridamol given twice a day, 5 days/week, resulted in a 24% increase in CD of young rat heart. These findings were confirmed by Hudlicka et al. (1983) on rabbit hearts chronically treated with adenosine and HWA 285 (a methyxanthine

derivative). These findings indicate that increased blood flow per se imposes mechanical stimulation on capillary endothelium and stimulates capillary proliferation.

2.c. Long-term bradycardial pacing:

Coronary blood flow changes during a cardiac cycle with diastolic CBF higher than systolic CBF. Thus whenever there is a decrease in heart rate, diastole is prolonged and so is the duration of diastolic perfusion. This could favor blood flow-induced capillary growth. Resting bradycardia has been reported after prolonged physical training (Tomanek, 1970). Furthermore, several studies have demonstrated an increase in myocardial CD and decreased heart rate in trained animals (Bloor and Leon, 1970; Tomanek, 1970; Crisman et al., 1985), as well as in naturally athletic animals such as the hare and wild rats (Wachtlova et al., 1965 and 1967), when compared to sedentary control animals. Furthermore, Lund and Tomanek (1978) showed a decline in CD of SHR rats in association with tachycardia. These findings suggest that increased diastolic CBF during bradycardia may play a role in stimulating capillary growth. In fact, Wright and Hudlicka (1981) showed convincing evidence in support of this hypothesis. These investigators implanted pacing electrodes in the right atrium of rabbits and cut the heart rate by 55% of normal up to 52 days. This caused a dramatic increase in CD of paced animals, which was not associated with hypertrophy indicating real capillary growth. However, CBF was not measured, which would have been helpful to see whether increased CD is associated with a concomitent increase in CBF.

To recapitulate, there is strong evidence to support the notion that, besides any chemical factor(s), increased CBF per se, such as during exercise, bradycardia and prolonged pharmacologic vasodilation can stimulate capillary growth in the mammalian heart.

V-4. Suggested studies:

The findings presented in this thesis can lead to a series of studies which we consider important for a better understanding of coronary physiology and myocardial performance during normal development. There are at least four categories of studies branching from these findings that can be performed.

4.A.: Studies on intact animals:

As mentioned earlier in this chapter, that one limitation of using the isolated, crystalloid-perfused heart preparation in this study, is our lack of knowledge to the extent that these results can be applied when the heart is intact and blood-perfused. Techniques are available to measure CBF in conscious rodents (Wangler et al., 1982; Peters et al., 1984). It would be worthwhile to make use of these techniques, and test whether these age-related changes can be shown in the intact animal. Furthermore, it will be interesting to expand such studies to include animals of older ages, ie. to examine whether CVDR changes between middle-age and senesensce.

4.B. Functional consequences of the decreased CVDR:

The consequences of the decreased CVDR need to be extensively studied. For example, the influence of the decreased CVDR on solute

exchange between blood and myocardial interstitial fluid should be examined. The decreased CD and decreased coronary flow reserve may limit not only nutrients' delivery, but their blood-cell transport as well.

It is also interesting to study the modulating influences of diet and certain kinds of age-related diseases, such as coronary artery disease and hypertension, on the age-related decline in coronary vasodilator reserve presented in this study.

4.C. Mechanism(s) underlying increase coronary vascular resistance and myocardial performance:

Studies are needed to elucidate the mechanism(s) underlying the increased vascular resistance reported in this thesis. Under this category we suggest the following studies:

1) It is unknown whether the increased vascular resistance reported here is due to a decreased CD or a decrease in other microvascular segments such as the small arterioles. Further histological studies are suggested to evaluate this point.

2) As mentioned earlier, there is a possible role of impaired relaxation mechanism of the vascular smooth muscle on the subcellular level, which may account for the increased coronary vascular resistance with age. To test this hypothesis, there are at least two areas to be studied.

a) Ca^{++} removal from cytosol of vascular smooth muscle as has been done for cardiac muscle by Froehlich et al. (1978).

b) the phosphorylation and dephosphorylation processes involved in the actin-myosin interaction. For example, a delayed rate

of dephosphorylation of the acto-myosin complex would delay the relaxation process.

3) Cocks and Angus (1983) have demonstrated that NE stimulates the release of an endothelial alpha-2-mediated relaxing factor in the rabbit aorta. Recent evidence has shown that the population of this type of receptor declines during the early stages of development in the aortic, mesenteric and renal arteries of rabbits (Tayo et al, 1985). Therefore, there is a possibility that a similar change occurs in the coronary vessels and contributes to decreased vascular relaxation.

4) The results presented in this thesis showed that maximum coronary flow per unit mass decreases with development until the age of sexual maturity. At this age, several hormones, in particular sex steroids, somatotropic hormone and thyroxine play important roles in several physiological processes. Hudlicka (1984) reported that cortisone, adrenocorticotropic hormone and estrogen have an inhibitory effect on angiogenesis, whereas progesterone and other luteal factors accelerate vascularization. Thus it is likely that the changes in hormonal pattern during development are somehow related to myocardial vascularization as well. Studies are needed to investigate the role of these hormones in myocardial vascularization.

5) We have demonstrated that, in response to NE, adenosine release is enhanced in the middle-aged guinea pig heart. Adenosine is known to have a negative inotropic and negative chronotropic effects on the heart. It is also known that cardiac inotropic and chronotropic responses to catecholamines are decreased with increasing age. We propose that the increased interstitial concentration of ado reported in this study may, at least in part, account for the decline in

chronotropic and inotropic responses to catecholamines with increasing age. This hypothesis can be tested by simultaneously infusing the ado degrading enzyme adenosine deaminase, along with NE to the middle-aged heart. Higher values on the chronotropic and inotropic responses in the treated heart would support the hypothesis.

4.D.: Can the decreased cardiac performance with age be reversed?

It was mentioned earlier that cardiac performance decreases with age. The exact mechanism(s) underlying these changes are still illdefined. The results presented in this thesis showed that coronary flow and oxygen delivery to the older hearts are reduced during increased myocardial performance when compared to the young hearts. Whether the decreased performance is the result of the gradual decrease in coronary flow and O_2 delivery is unknown. What is known, however, is that chronic exercise prevents the well documented decline in capillary density with age (Tomanek, 1970). Furthermore, Spurgeon et al. (1983) showed that chronic exercise also prevents the increased contraction duration and increased peak developed tension characteristic of increasing age. The idea of the decreased performance due to decreased blood flow is an old one. Leonardo da Vinci wrote, on the basis of his dissections, that the cause of aging was "veins which by the thickening of their tunics in the old restrict the passage of blood, and by this lack of nourishment destroy the life of the aged without any fever, the old coming to fail little by little in slow death" (cited by Belt, 1952).

Therefore, studies are needed to test whether the decreased cardiac performance is related to the decline in coronary vasodilator reserve (CVDR) reported in this thesis. Such studies could include attempts to

reverse the decrease in CVDR by increasing CD such as by exercise, long-term bradycardial pacing, or chronic pharmacologic vasodilation, and correlate these to cardiac performance.

In conclusion, there are several ideas that can be derived from the results and information presented in this thesis, to provide a life-time of hypotheses remaining to be tested.

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