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Abstract

Cardiac output, its distribution and organ blood flows were measured with radiolabeled microspheres in young adult (5 months old), middle aged (12 months old) and senescent (24 months old) Sprague Dawley rats. Heart rate and cardiac output did not change with age but the distribution of cardiac output is markedly affected by the age of the animal, most notably a gradual decrease in both the renal and splenic fractions of cardiac output. Other organs including the liver, stomach and brain, show a rise and then a fall in their fractions. Only the renal blood flow, normalized for weight, shows a definite age related trend, decreasing to about half the young adult value in the oldest group.

Administration of equivalent doses of isoproterenol to these rats produced a spectrum of effects in the three age groups. The youngest group showed the strongest responses in increased heart rate, cardiac output and cardiac output normalized for body weight. Only the heart rate increased significantly in the oldest group. The coronary, skin and carcass blood flows increased after isoproterenol in the youngest age group. Only the right ventricular blood flow was elevated, while the renal and small intestinal blood flows fell in the middle age group. No significant redistribution of cardiac output was detected in the senescent age group. It is concluded that resting

cardiovascular values do not change significantly with age, with the exception of the renal blood flow. In contrast, the response to adrenergic stimulation is markedly influenced by the age of the animal.

Similar measurements were made in 5 months old and 10 months old Lewis f/Mai rats. Equivalent doses of pentobarbital decreased stroke volume, cardiac output and cardiac output per kilogram in the ten months old rats but not in the five months old rats. The renal and hepatic fractions and flows increased greatly in both age groups after pentobarbital. Only the carcass blood flow fell in the younger group, but almost all organ blood flows in the older group fell after pentobarbital. It is concluded that pentobarbital anesthesia profoundly affects the older cardiovascular system, probably through the mechanism of myocardial depression as well as through direct effects.

Heterotopically transplanted hearts in the above animals, received 17 % to 29 % of the fraction of cardiac output that the recipient hearts received in the two age groups. Blood flows per gram were 34 % and 38 % of the host coronary blood flow. The above values do not differ significantly with age.

It was shown that special attention must be given to measuring parameters under truly basal conditions. As presented here, beta agonist effectiveness decreases with age and the adverse cardiovascular effects of pentobarbital increase with age.

The importance of intermediate age groups in research on aging was also shown. Several examples of transient changes in organ fractions of cardiac output or blood flows were discovered that otherwise would have been missed.

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Chapter 1

Introduction

1.1

The Effect of Age on Cardiac Output and its Distribution

Cotchin and Roe (1967) and Yates and Hiley (1979) both note a need for more complete information on the physiological and pathological effects of ageing in experimental animals. Physiologists, pharmacologists, toxicologists and other biological scientists would benefit from a sound understanding of age changes in the normal organism. Clinicians, especially those in the gerontological subspecialties and of course their patients, would gain much from more complete knowledge of the normal changes associated with ageing and of the mechanisms involved. Unlike some areas of basic research, studies on ageing have, to the layman and the professional alike, readily apparent applicability in the enhancement of the quality of our lives.

In view of the importance of research into the effects of ageing mentioned above, effort is now focussed on investigation of the senescent cardiovascular system. Surprisingly, while reviewing the literature, I found a paucity of data on blood flows in the senescent heart, and in other organs as well.

Changes in regional blood flows as determined from measured cardiac output, and the fractional distribution of

cardiac output, are well described in the early stages of life in the rat (Rakusan and Marcinek 1973; Stulcova 1977 for example), but little data are available past the stage of young adulthood. Studies on the blood flow of some individual organs are relatively common, but only Bender (1965) has considered the literature on senescent organ blood flows in terms of changes in the overall pattern of blood flow; that is, the distribution of cardiac output. His conclusions were that total peripheral resistance is increased and systemic blood flow is reduced with age. Cerebral, coronary and skeletal muscle flows are favoured at the expense of liver and kidney blood flows in the face of the decreasing cardiac output. These observations have not been confirmed in animals. I therefore decided to measure cardiac output and its distribution in "young adult", in "middle aged" and in "senescent" laboratory rats under identical experimental conditions.

Vizek and Albrecht (1973) found that cardiac output measured at intervals with the dye dilution method in anesthetized rats does not change from fifty days to four hundred days of age. Cardiac output normalized for body weight decreased, from 450 ± 23 ml/min·kg at thirty days of age, to 284 ± 35 ml/min·kg at sixty days to 178 ± 24 ml/min·kg at four hundred days. Popovic and Kent (1964) found that cardiac output did not change in chronically cannulated conscious rats of 300 grams initial body weight over 120 days.

In a paper by Lin and co-workers (1970) it was noted that cardiac output per kilogram in small animals decreased with increasing weight (age) as measured by various authors using the Fick, dye dilution, radioisotope dilution or thermodilution techniques. Lin and co-workers confirmed this in rats of 143 to 564 grams using the last technique.

Yates and Hiley (1979) found that cardiac output did not change in anesthetized rats from 3-4 months to 11-12 months old rats, but that cardiac output per kilogram is less in the older group. Using a dye dilution method, Morvai and Ungvary (1979) found that cardiac output increases from 2 months old rats to 26-28 months old rats, but that cardiac output normalized for body weight decreases.

In Man, Brandfonbrender and associates (1955) and earlier workers found that resting cardiac output in normal men decreased with age over the period from 20 to 90 years. Amery and colleagues (1978) found that cardiac index falls with age in Man due to a decline in stroke index and not heart rate.

Cardiac output per kilogram in children of less than 14 years was found by Katori (1979) to be higher than that of people older then 15 years. Absolute cardiac output increased with age up to 15 years and then remained constant, but cardiac output per kilogram decreased with age in persons older then 20 years.

The increase in cardiac output with age appears to result from increasing mass. After young adulthood cardiac output normalized for body weight or surface area generally declines with increasing age.

The distribution of cardiac output at different ages has been measured by various investigators. Rakusan and Marcinek (1973), using the Sapirstein method (Sapirstein 1958), measured the distribution of cardiac output in newborn, 1, 2 and 5 month old conscious rats. Coronary and bronchial fractions of cardiac output are high in newborn animals and decrease rapidly to adult values. In contrast, the renal fraction is low in newborns and increases with age.

In 1977 Stulcova, using microspheres of 15 μm in diameter, measured the distribution of cardiac output in rats aged 9, 18, 25, 42 and 64 days old under ether anesthesia. The microsphere method will be more fully described later in this introduction. As in the previous study, the coronary fraction decreased and the renal fraction increased with age.

In a study of 17-60 days old anesthetized rats also using 15 μm in diameter microspheres, Aperia and Herin (1975) found that the kidney fraction of cardiac output increased continuously from 17 to 60 days and that renal blood flow, normalized for kidney weight, shows a slow increase up to 30 days and then a faster increase thereafter. Renal blood flow per gram reached adult levels

by about this time.

The distribution of cardiac output and organ blood flow in two months old and twenty six to twenty eight months old rats was measured by Morvai and Ungvary (1978) using the older Sapirstein method of following the fractional distribution of a radioactive indicator. These rats had been placed on a liquid diet for four weeks, as they were controls in a study on alcohol intake. The fraction of cardiac output to the gut and to the brain decreased with age, while the carcass fraction of the cardiac output increased. Blood flow per gram of organ weight decreased in the brain and the skin.

Using microspheres, blood flows per gram were found by Yates and Hiley (1979) to decrease in skin, skeletal muscle, splanchnic organs and kidneys, in 3-4 months to 11-12 months old anesthetized rats.

When measured by diodrast clearance, Davies and Shock (1950) found a 53 % decline in effective renal plasma flow, normalized for body surface area, in 20 years old to 90 years old men. The decline was approximately linear beyond 30 years of age. Supporting this observation, Hollenberg and co-workers (1974) found a significant age related renal blood flow reduction in men of 17-76 years of age, and suggested renal vascular changes as the reason for this decline.

Amery and co-workers (1978) also reported a 50 % reduction in human renal blood flow between 40 and 85 years,

which they suggest is due to a reduction in the number of functioning nephrons.

1.2

The Effect of Stress on the Senescent Cardiovascular System

Textbooks on pharmacology and anesthesiology, if they mention geriatrics at all, emphasize that the therapeutic margin in older people is often less then that in younger ones. Careful titration of the dose or treatment to each patient is recommended but usually no quantitative instructions are given. In the response to a variety of stimuli, younger individuals have a reserve upon which they can call, that is reduced or absent in older individuals.

The response of the senescent rat cardiovascular system to various types of stress seems to be different from young adult responses. Gerstenblith and associates (1976) suggest that ageing impairs the cardiovascular response to exercise in almost every parameter measured, including maximal heart rate, stroke volume and A-V oxygen difference. Chiueh and co-workers (1980) noted total cardiovascular collapse after 30 minutes of forced immobilization in old Fischer-344 rats and not in younger ones. Lakatta (1980) reported that decreased response to beta adrenergic stimulation may partially explain the diminished cardiovascular responses to acute stress, but more information is needed.

The attention payed to experimental conditions in

the results quoted in this thesis was intentional. In the past, investigators of the effects of ageing have used a variety of preparations, including anesthetized, restrained, stressed and relatively unstressed animals. In view of the largely unknown changes with age in the cardiovascular responses to various stresses, the interpretation of these results is almost impossible.

In order to be able to synthesize the results of investigators into a general description of senescent changes in the distribution of cardiac output, I decided it was first necessary to examine some cardiovascular effects of stress at different ages. The following types of stress were briefly considered.

1 Traumatic stress, such as that produced by the Noble-Collip rotating drum (Malik et al 1978) was considered to be too variable for our purposes and the additional factor of age related effects of anesthesia on the parameters measured would have to be accounted for. Study of the effects of trauma at various ages was not of primary interest here, and its use could be considered unethical in this instance, as would other intense mechanical or thermal interventions that have been used in the past.

2 Hypovolemic stress (Blahitka and Rakusan 1977) was rejected because of the probability of an unacceptably high mortality rate in the older groups.

3 Exercise is a more physiological stress to the cardiovascular system. Age related alterations in the

cardiovascular effects of ex ercise have been reported (Strandell 1964; Montoye et al 1968; Gerstenblith et al 1976). The measurements that were proposed for this study require the use of two implanted cannulae. It was thought that strenous exercise and the inevitable jostling of the animals would produce too many technical problems to be practicable.

4 A stress induced by catecholamine treatment was considered, as sympathetic activation is a common denominator in different types of stress. Catecholamines were chosen because of the vast amount of information that has already been gathered on their actions.

I wanted to examine the effects of two common laboratory stresses at different ages. The first was a simulation of sympathetic nervous system activation. In order to standardize the stress, chemical stimulation was decided upon. Catecholamine infusion was used here.

The second stress studied was anesthesia. The cardiovascular effects of pentobarbital administration in rats are known but the influence of age on these effects is not.

The next sections will deal with the mechanics of the simulation of sympathetic stimulation as used in this investigation. Known effects of isoproterenol and age related changes of these effects are also discussed.

1.2.1

Choice of Isoproterenol, Dose and Route of Administration

Isoproterenol is a synthetic catecholamine. It was chosen for the following reasons. Isoproterenol affects almost every vascular bed in the body. Age changes in the vascular and metabolic response of major organs to beta adrenergic stimulation could have profound effects on the distribution of cardiac output. Since isoproterenol is a pure beta agonist, interpreting its effects should be more straightforward then interpreting a mixed receptor activating drug.

The effects of isoproterenol on circulatory hemodynamics in young rats is known (Kapitola et al 1973). It has also been shown that some responses to beta adrenergic stimulation are altered with age (Charbon and Reneman 1970; Fleisch et al 1970; Debreczeni and Fenyvesi 1971; Fleisch 1971, 1980; Gulati et al 1973; Lakatta et al 1975; Fleisch and Hooker 1976; Park et al 1976; Yin et al 1976, 1979; Imms et al 1977; Kuramoto et al 1978, 1979; van Brummelen et al 1981). These changes will be more fully discussed below.

Isoproterenol has been administered by the intraperitoneal (Kapitola et al 1973), subcutaneous (Stanton et al 1969) and intravenous routes (Schroeder et al 1970; Debreczeni and Fenyvesi 1971; Hoffbrand et al 1973; Debreczeni et al 1980).

Kapitola injected rats with 1 mg/kg isoproterenol IP and found that peak 86-Rb uptake in the heart occured at

approximately 30 minutes after injection. However, in another group at 4 hours after the injection, 86-Rb uptake was found to be below control values. The time course of the actions of isoproterenol administered IP is not well known. The IP and subcutaneous routes are used most often for studying cardiomegaly and isoproterenol induced cardiac lesions. Doses as high as 80 mg/kg SC are used for this purpose (Stanton et al 1969).

Hoffbrand et al (1973) used the much lower dose of 0.06-0.9 µg/kg·min IV isoproterenol for 20 minutes when studying dose related effects of isoproterenol in conscious monkeys. Schroeder et al (1970) found that heart rate, cardiac output and vasodilation responses to intravenous infusions of 0.05, 0.1, 0.25, and 0.5 µg/kg·min isoproterenol into dogs, reaches steady states between 1 and 2 minutes.

Debreczeni and Fenyvesi (1971) infused isoproterenol at 0.3 µg/kg·min into rats and found that blood pressure, after a slight fall, stabilized within five minutes. After five minutes of isoproterenol infusion into rats, Debreczeni and co-workers (1980) found that at doses above 0.1 µg/kg·min cardiac output and some organ blood flows differed significantly from control values.

Hemodynamic values in this study were therefore also measured after 3-5 minutes of 0.2 µg/kg·min. IV infusion of isoproterenol.

1.2.2

Age Changes in Cardiovascular Actions of Isoproterenol

A principle action of isoproterenol is its effect on vascular beds, namely vasodilation. This occurs by a direct effect on the vessels and possibly by baroreceptor reflex activation (Schrier 1974). In most tissues it reduces vascular resistance to blood flow, allowing more blood through, increasing venous return and cardiac output (Cobbold et al 1960; Shanks 1966; Schroeder et al 1970; Greenway and Murthy 1972; Kapitola et al 1973; Hoffbrand et al 1973; Green 1977; Imai et al 1978). It has positive inotropic and chronotropic effects on the heart which further increases cardiac output. Diastolic pressure decreases while systolic pressure may remain unchanged or decrease slightly (Meyers et al 1978). Charbon and Reneman (1970) showed regional differences in isoproterenol sensitivity in unanesthetized dogs. The coronary and the coeliac vascular beds were the most responsive, measured by electromagnetic flowmeter.

Age related changes of the cardiovascular response to sympathetic stimulation have been reported. Fleisch and co-workers (1970) and Fleisch (1971) reported an age dependent decrease in rat aortic sensitivity (relaxation response) to isoproterenol in rats of different strains as well as a gradient of sensitivity of beta receptors from the thoracic to the abdominal aorta. Tuttle (1966) reported a decrease in the sensitivity of rat aortic strips to

catecholamines, both in threshold and magnitude. Rats used were three months to one year and two years old. Fleisch and Hooker (1976) demonstrated this effect in rabbit and rat pulmonary arteries.

Ericsson and Lundholm (1975) also found in aortic strips from six months old rats, decreased relaxation responses to isoproterenol compared to aortic strips from one month old rats.

Hayashy and co-workers (1977) demonstrated an age related decrease in beta mediated relaxation in isolated rabbit basilar arteries constricted with histamine. Fleisch and Spaeth (1981) reported that isoproterenol produces less relaxation in three months and six months old perfused rat mesentery, already constricted with noradrenaline, than that produced in three weeks old rat-mesentery. They concluded that beta receptor activity in resistance vessels decreases with increasing age.

Veins do not appear to lose functional beta receptors with age (Fleisch and Hooker 1976; Fleisch 1980). Altura and Altura (1977) showed that the relaxation of mesenteric venules from young, mature and old rats in response to isoproterenol was independent of age.

Yin et al (1976, 1979) reported an age associated decrease in chronotropic response to isoproterenol both in Man and in beagles. Baseline heart rate in young and old age groups in both Man and Dog did not differ. They suggest that the age difference is due to a decreased number of

atrial chronotropic beta receptors. The heart rate increase after isoproterenol administration was not limited by atrial responsiveness since electrical pacing could increase heart rate further in both young and old dogs. Cholinergic inhibition does not play an important part because atropine did not block the difference in response between the two age groups.

1.2.3

Mechanisms Proposed for Age Related Changes of Effects of Isoproterenol

Lakatta and associates (1975) and Guarniere and co-workers (1980) demonstrated that the contractile response to catecholamines in the myocardium (dT/dt) is diminished with age. The latter study also indicated that receptor affinity and number, in the myocardium, does not change with age. They propose that whatever the inhibitory factor(s) in muscle contraction in the senesencent myocardium is, it acts subsequent to the protein kinase activation step but proximal to the Ca++ troponin interaction step.

In a recent paper, Abrass and co-workers (1982) found no change in beta-adrenergic receptor number or receptor affinity in rat hearts, lungs or lymphocytes. They did find the chronotropic response to isoproterenol to decrease with age. This substantiates the above interpretataion of an inter-cellular deficit in response to beta stimulation, rather than a change in receptor

characteristics.

Decreasing beta receptor number and concentration with age in lymphocytes from human males 24 to 81 years old was found by Schocken and Roth (1977). Receptor numbers fell from 14,000 to 8,000 sites per cell. Perhaps an analogous decrease in smooth muscle beta receptor concentration is responsible for some of the age related changes in the vascular response to isoproterenol. Greenburg and Weiss (1978, 1979) found a decreased number of beta receptors in homogenates of the old rat brain.

Decreasing hormone receptor concentration with age was found by Roth in a review to be the most commonly found manifestation of ageing at this level (1979). In contrast, the receptor-hormone affinities he examined were found not to change with age.

Fleisch (1980) also suggests a decrease in the number of beta receptor sites. Other mechanisms that may be responsible involve either reduced binding of agonists to receptors, or decreased vascular sensitivity to cyclic nucleotides.

Kelliher and Conahan (1980) point out that their results with vagotomy and muscarinic receptor stimulation support the notion of a generalized reduction in all autonomic control of heart rate with age. Heart rates decreased from three months old anesthetized F-344 rats, through twelve and to twenty four months old animals. The increase in heart rate they found after vagotomy was less

pronounced in older animals. They thought this evidence for the "loss of receptor" theory.

An apparent age related decrease in response to isoproterenol could be produced if the isoproterenol was broken down more rapidly in older subjects. Isoproterenol, unlike the natural catecholamines, is not a substrate for the enzyme monoamine oxidase. It is biotransformed into an inactive form by the widely distributed enzyme catechol-o-methyl transferase (COMT). In the heart, kidney and brain however, COMT activity is relatively unchanged over the life span of animals tested (Gey et al 1965 and Prange et al 1967).

A factor opposing the uncovering of any clearcut age dependent blunting of the beta adrenoceptor responses in vascular smooth muscle (vasodilation), is the fact that isoproterenol facilitates noradrenaline release through stimulation of presynaptic beta adrenoceptor sites (Langer 1976). This action has been shown in some animal tissues (Stark 1977) and in human arteries and veins by Stjarne and Brundin (1976). If presynaptic and postsynaptic beta adrenoreceptor site effects decline with age, this would obscure age related differences in isoproterenol induced vasodilation by greater facilitation of noradrenaline release in younger subjects (van Brummelen et al 1981). The fact that any age dependent decline is seen at all indicates that in some tissues, vasodilatory effects must predominate; that the pre- and postsynaptic beta adrenoceptor activity

decline does not occur at the same rate, or that in some tissues, isoproterenol facilitated release of noradrenaline is absent or unimportant.

Lakatta in his 1980 review indicated that in man, diminished responses to stress are not due to a failure to secrete catecholamines. He reviews recent findings concerning adrenergic stimulation in different age groups and concludes that all proposed mechanisms are compatible with the interpretation that the effectiveness of adrenergic stimulation declines with age.

The preceding discussion points to an age related decline in the response to beta adrenergic stimulation through any of the following mechanisms: a decrease in receptor number, increased agonist breakdown, uneven balance of vasoconstriction and vasodilation response to beta adrenergic stimulation, or theoretical intracellular inhibitors of vascular smooth muscle contraction.

In sharp contrast to the above studies some published observations point to an increase in the senescent response to catecholamines. Bender (1970) reviews several studies indicating increased sensitivity or responsiveness with age in contraction of cat nictitating membrane and excitation of cerebral structures by catecholamines. The LD 50 of epinephrine decreases by 3/4 in mice from 5 to 16 months of age. He cites studies showing age related decreased excretion of catecholamines due to the loss of renal function and delays in absorption of catecholamines

from their site of administration.

Equivalent doses of adrenaline or noradrenaline increase the blood pressure more in aged rats than in young ones (Hruza and Zweifach 1967). Threshold doses for producing this elevation of blood pressure are lower in older animals (Frolkis 1979). This increased sensitivity, "supersensitivity" according to Lakatta (1980), is in marked contrast to the more generally accepted results reported in the preceding paragraphs, namely decreased responsiveness. A supersensitivity to catecholamines has been described in the catecholamine depleted heart (Cooper 1966). The 25 % reduction in catecholamine content found in senescent hearts is not enough to cause this denervation supersensitivity-like phenomenon. Weiss and co-workers (1979) found that the brain in aged rats has a diminished capacity for developing increased numbers of beta receptors in response to a reduced sympathetic input. The blood pressure elevation due to "catecholamine supersensitivity" is more probably the result of mechanical changes in the stiffness of the arterial system rather than changes in beta receptor characteristics.

There is no doubt that there are age related differences in the autonomic response to stress. Beta receptors in particular are affected by age. Tissues are supplied with different combinations of the adrenergic receptor types. Basal blood flows through different areas may be affected by asynchronous age changes in sensitivity

to beta agonists. Blood flow in older animals must especially be affected in times of stress with both the distribution of cardiac output and normalized organ blood flows differing from those in stressed young animals.

1.3

Effects of Pentobarbital on the Senescent Cardiovascular System

Since pentobarbital is so commonly used for small animal anesthesia, and since its cardiovascular effects at different ages are not known, it was decided to investigate this hypnotic in rats of different ages.

Forsyth and Hoffbrand (1970) investigated the effect of pentobarbital anesthesia on cardiac output distribution in monkeys. Kaihara and co-workers (1968), using microspheres, examined the effect of sodium pentobarbital in dogs. Both authors found increased lung and liver fractions of the cardiac output. Popovic and Kent (1964) found that pentobarbital decreases cardiac output in the chronically cannulated rat. Sasaki and Wagner (1971) found a decreased brain fraction of the cardiac output and increased lung, spleen, pancreas and gastro-intestinal fractions after pentobarbital anesthesia in rats. Rakusan and Blahitka (1974) found decreased fractions of cardiac output to the heart and brain, and increased fractions to the kidney and skin. Aardal and co-workers (1973) found a similar pentobarbital induced redistribution of cardiac

output from the skeletal muscle to the kidney and intestines in rabbits.

Since many earlier age related investigations were performed in anesthetized animals, and the effect of anesthesia on blood flows at different ages has not yet been evaluated, it was deemed prudent to do this. For proper interpretation of the work of others investigating age changes, knowledge of the effect of experimental conditions including anesthesia is necessary.

1.4

Measurement of Organ Blood Flows

As emphasized above, conditions under which the experiment is done may have adversely affected the results of age related investigations. The method used to measure the distribution of cardiac output can also affect conclusions drawn when comparing studies, and thus assumes greater importance than usual. It is for this reason that a discussion of the techniques used for measurement of the distribution of blood flow is given here.

1.4.1

Direct Methods

Several methods have been developed to determine the amount and distribution of blood flow in experimental animals and man. The most straightforward is sectioning the vessels emptying a tissue in question, and collecting the

venous outflow over time. The obvious limitations of this technique make it unacceptable for use in man and in the animal model of ageing used here.

Electromagnetic flowmeters have been used to study blood flows in vivo (Charbon and Reneman 1970 for example). Surgical exposure of a vessel is necessary. In the rat, chronic measurement of blood flow is limited to only a few organs. No indication of blood flow distribution can be obtained other than that through the major arteries directly measured. Drift of the calibration can also complicate interpretation of data collected hours or days apart. This method is used most often when continuous monitoring and analysis of transitional flows is required.

Doppler measurement of blood flow is still too unrefined a procedure to consider its use in rats.

Determination of blood flow by following the washout of radioactive indicators (133-Xenon) has only limited applications in research on small animals and is not suitable for multi-organ studies (Kety 1951). There are still questions about the validity of this method that have yet to be satisfactorily answered (Marcus et al 1981).

1.4.2

Sapirstein's Method

The distribution of cardiac output has been estimated by measuring the tissue uptake of radioactive potassium and later by using a potassium analogue,

86-Rubidium (Sapirstein 1958). Uptake of 86-Rb from the blood by an organ is assumed to be proportional to its perfusion over a 30-60 second period, at which time the animal is sacrificed and the organ radioactivity measured.

For this method to work, the ratio of 86-Rb removed from the blood by each organ to the amount recirculating, the "extraction ratio", is assumed to be identical to the extraction ratio of the whole body. This assumption has yet to be validated in some organs. Tritiated water has also been used (Tripp et al 1977) instead of 86-Rb as the indicator. Although some assumptions for the latter technique differ from 86-Rb use, its validity is still questioned for the reasons given below.

Measurement of blood flow to organs by uptake of a radioactive indicator (86-Rb) is affected by the rate at which individual organs absorb the indicator. For example, the brain cannot be measured with 86-Rb as its extraction ratio has been found not to be constant after injection of the isotope (Sapirstein 1958). Iodoantipyrine is used to measure brain blood flows instead. Knoebel and co-workers (1978) presented evidence that the rate of coronary uptake of Rubidium is identical to that of the whole body. However Rakusan and Blahitka (1974) and Foster and Frydman (1978) both found differences between the coronary fraction of cardiac output calculated with this method and that calculated by the microsphere method, as discussed below.

Organs with a dual capillary bed or blood supply (kidney, liver, lungs) may be overestimated as well as organs with sluggish blood flows (Tancredi et al 1975). The Sapirstein method for determining the distribution of cardiac output (Sapirstein 1958), as shown by Rakusan and Blahitka (1974) overestimates the kidney fraction. These authors also found an increase in the kidney fraction due to anesthesia. Mendell and Hollenberg (1971) were the first to report differing results between the microsphere and the Sapirstein methods. They found that the heart, brain and spleen fractions of cardiac output were higher and the liver fraction lower when measured with microspheres than results obtained with the Sapirstein method.

A large body of literature using the 86-Rb technique has accumulated however (1958-1967 and even later), before these problems came to light. Its advantages over the microsphere technique, described next, is in its simplicity, as only a venous cannula is needed, and its cost is lower. Major drawbacks of this method are that it can only be performed once on the same animal, almost immediate killing is necessary, and its validity rests on several still unproven assumptions. Most authorities now believe that the distribution of blood flows should be measured with other, more accurate methods (Marcus et al 1981).

Sapirstein's method produces consistent results and can reflect differences in the distribution of blood flows (Mendell and Hollenberg 1971). The relative fractions

of cardiac output for the brain, liver, heart, spleen and kidney, may however, not match those of other independent methods. The reliability of this method decreases with very high and very low blood flows. High flow rates may lead to underestimation and low flow rates may result in overestimation of blood flows (Foster and Frydman 1978). Nevertheless one can find many examples of the detection of trends in organ perfusion that have later been shown to be accurate.

Vizek and Albrecht (1973) examined blood flow changes in anesthetized rats of 48 and 65 days using Sapirstein's method of fractional distribution of 86-Rb. They found that gastrointestinal tract and skin blood flows declined during this period.

Reported values of the various fractions of the cardiac output and organ blood flows normalized for weight differ, reflecting both the drawbacks of the Sapirstein method and different experimental conditions. The kidney fraction of cardiac output for example is 7.6 ± 0.7 % in two months old anesthetized rats measured by the microsphere method (Stulcova 1977) but 15.5 ± 0.5 % in similarly aged conscious rats (Rakusan and Marcinek 1973) and 19.5 ± 1.2 % in two months old rats after sodium pentobarbital anesthesia (Morvai and Ungvary 1978), both measured with the Sapirstein method.

Comparison of an organ's fraction of cardiac output at different ages is difficult because organs can

exhibit growth which is disproportionate to that of the whole body. An apparent increase with age in an organ's fraction of the cardiac output may only reflect an increase in the relative size of the organ. When an organ's fraction of cardiac output is expressed as the % of cardiac output per relative body weight, a weight independent index is obtained. An organ perfused with a fraction of cardiac output concomitant to the perfusion of the whole body would have a relative flow of 1.0. The kidney fraction in rats at two months, expressed with this index is 10 ± 0.9 when measured with microspheres (Stulcova 1977), but 15 ± 0.5 measured with 86-Rb (Rakusan and Marcinek 1973).

At one month of age, according to Rakusan and Marcinek, using 86-Rb, the organ with the highest relative flow index is the kidney (9.2), followed by the heart (4.3), small intestine (2.8) and then lungs and stomach (2.3). Stulcova, using microspheres, found a similar sequence. She was also able to measure the relative flow to the brain with this technique, obtaining the index of 2.1. This fraction is not measurable with the Sapirstein method. If relative weights do not change in the organs compared, the fractions of cardiac output alone may then be compared.

1.4.3

The Microsphere Method

This method was chosen as the most reliable technique available for accurate simultaneous measurement of

multi-organ blood flows. Labeled glass microspheres were used as early as 1948 to study organ blood flows (Prinzmetal et al 1948). The method involves intravascular injection of microspheres which are distributed in proportion to organ blood flows, and are trapped in terminal vascular beds anywhere they meet a limiting vascular diameter. The first rigorous examination of the use of this technique for the measurement of multi-organ blood flows was by Rudolph and Heymann (1967). An excellent review of the technique and problems associated with its use is given by Heymann and associates (1977).

Being a purely mechanical process, the microsphere method does not depend on the metabolic activity of each tissue. If the total number of microspheres injected is known, and a reference sample of blood and microspheres is withdrawn downstream from the site of the injection at a known rate, the cardiac output can be determined and organ flow rates calculated. To enable counting, the microspheres are labeled with a radioactive isotope. Microspheres labeled with different isotopes allow for multiple determinations. In order to obtain meaningful results several conditions have to be satisfied:

1. microspheres must be uniformly mixed with the blood after injection, with no streaming of the microspheres.

2. injection of microspheres and withdrawal of the reference sample should produce minimal hemodynamic effects.

3. the blood should be totally cleared of microspheres

after one pass through the circulation.

4. trapped microspheres should remain stable in each organ.

5. a minimum number of microspheres in each tissue measured must be met to avoid large sampling errors.

6. the total number injected must be known in order to determine organ fractions.

7. the method should correlate well with other methods and be reproducible.

1.4.3.1

Review of Studies Concerning the Accuracy of the Microsphere Method

The conditions listed in the preceding section are those thought necessary for the proper operation of the microsphere method. As this is the technique that I used, it is important to understand its limitations.

1 Mixing of Microspheres: Kaihara et al (1968) tested the adequacy of mixing by concurrent microsphere injections using two different isotopes. He reasoned that if mixing was adequate, the distribution of the two isotopes should be the same. He found no difference in distribution of blood flows in dogs when simultaneous injections of differently labeled 50 um microspheres were injected into the left atrium. When the injection site was changed from the left ventricle to the origin of the aorta, only the percentage of

microspheres found in the heart varied appreciably.

Buckberg et al (1971) also commented on the possible problem of inadequate mixing. In dogs and sheep, left atrial injection was shown to be superior to left ventricular injection when studying blood flow to the heart with microspheres 8-50 μm in diameter. They found no difference in reference blood sample counts taken from femoral or carotid arteries after left ventricular or left atrial injection, indicating good mixing, but a higher variability was noted in the first case. They found no difference in counts between right and left kidneys.

Wicker and Tarazi (1982) recently measured coronary, renal and cerebral blood flows in rats, using both left atrial and left ventricular microsphere injection sites. They found no difference in mean values but the variability in the measured coronary blood flow was lower in the left atrial injection site rats than in the rats injected in the left ventricle. They suggest that left ventricular injections are adequate for measuring flows in distal vascular beds but coronary blood flow measurements may require left atrial injection.

Foster and Frydman (1978) injected two differently labeled 15 μm microspheres conturrently in rats and looked at their distribution in various organs and the concentration of each isotope in two arterial reference samples taken at the time of injection. No significant difference between isotopes, site of withdrawal of reference

samples, or calculated cardiac outputs were found. In the organs measured, differences in concentration of the two isotopes injected were within a theoretical statistical limit due to random variation discussed by Foster and Frydman (1978).

Phibbs and Dong (1970) found no evidence of settling of microspheres up to 80 μm diameter in the circulation, but found that the large and heavy microspheres tend to flow along the center of an artery. This becomes important only when studying intra-organ blood flows where 'plasma skimming' may occur. Hales (1974) discussed the topic of microsphere distribution in the blood and again raised the point that some of the principles and problems of using microspheres to study the distribution of blood flows to organs are different than those encountered in studying flow within an organ. Yarger et al (1978), Clausen et al (1979) examining the kidneys, and Maxwell et al (1981) investigating the intestine, also supported this view.

Sasaki and Wagner (1971) showed that left ventricular injections of 50 μm in diameter microspheres indicated no differences between measured blood flows in the right and left brain and took this to indicate adequacy of mixing. Forsyth et al (1968) found no difference in flows to each half of 19 monkey brains, or to the right and left kidneys with microspheres of 50-55 μm in diameter. Idvall (1979), using 15 μm microspheres, found equal flows to the right and left kidneys and a good correlation between

coronary blood flow and cardiac output after special preparation of the heart, which indicated to him adequate mixing.

2 Hemodynamic Effects: The condition that the method should have no significant effect on circulatory parameters must be satisfied. Warren and Ledingham (1974) using 15 µm diameter microspheres found no change in the distribution of cardiac output between two successive injections, but found significant changes with larger microsphere sizes, including a rise in the skin fraction of cardiac output and a fall in the kidney, gut, spleen and heart. Transient falls of cardiac output and blood pressure, bradycardia and hypertension were also noticed with larger sizes. The authors stressed that they did not see these changes with the smaller microspheres.

Foster and Frydman (1977) found no significant change in the distribution of cardiac output between repeat doses of 15 µm microspheres. It seems that the size of the injected microspheres is a factor in the hemodynamic perturbations produced by this method.

Roth et al (1970) reported nystagmus, tonic forepaw contractions and salivation in dogs following injections of 50 µm microspheres. Hales (1974) reported similar reactions using spheres of 80 or 175 µm and only very occasionally with smaller microspheres. Sasaki and Wagner (1971) using 50 µm microspheres, found no difference

in blood flow distribution measured from three hours to several days apart except for raised kidney fractions. It is important to note that this group commented on swelling of the paws of rats after injection. The microsphere suspension that they used contained 10 % dextran (a glucose polymer) used to extend settling time of the microspheres. It has been shown that dextran (Voorhees et al 1951) and not the microspheres themselves (Flaim et al 1978) have significant effects on blood pressure, heart rate, peak left ventricular systolic pressure and left ventricular end diastolic pressure, producing severe hypotension in rats. Swelling of the paws and face were also mentioned by the latter author as an effect of dextran.

Tsuchiya et al (1977) suggested an upper limit of 100,000 microspheres injected per rat, based on hemodynamic effects observed with larger numbers, but his suspending medium contained 10 % dextran. Flaim et al (1978) showed that up to 850,000 small 15 μm in diameter microspheres in saline alone could be injected into a rat without altering blood pressure. This large number is more than adequate for determining the distribution of cardiac output.

Tween 80, a detergent used to inhibit aggregation of microspheres, has small observable effects on rat hemodynamics. Flaim et al (1978) found a significant but small reduction in heart rate when Tween and saline were injected into rats. In another series of experiments, Tween, saline and microspheres produced no detectable

changes. Ishise and co-workers (1980) found no change in heart rate, arterial pressure, cardiac output or total peripheral resistance after three injections into rats of 15 μm microspheres at 30-36,000 per determination in saline and Tween 80.

Several authors report transient non-specific changes in ECG, heart rate and blood pressure at the time of microsphere injection (Ishise et al 1980; Forsyth et al 1968). These minor changes resolve themselves within seconds.

The use of microspheres of 15 μm in diameter, less then 850,000 per rat and suspended in saline, should satisfy this criterion of minimal hemodynamic effects. The rate of withdrawal of the reference sample should be low enough to not disturb the parameters measured.

3 Clearance of Microspheres: Blood should be cleared of microspheres in the first pass through each organ. However, the underestimation of one organ's blood flow by some fraction of the microspheres passing through it will not distort the general pattern of flow unless the total blood flow through the organ is high, or if the fraction of microspheres not cleared from the blood on the first pass is high.

If the end of the cannula used for microsphere injection is positioned in the aorta, the cephalic region and coronary blood flow may be underestimated.

Overestimation can occur: a) by aggregates of microspheres which lodge at random in an organ; b) by shunting or incomplete clearance by some organs; c) in the case of the bronchial flow by intravenous, or trans septal right ventricular injection; d) in the case of the heart by intramuscular injection or injection into the coronary ostia; and e) in the case of the carcass fraction by intrapleural, peritoneal or mediastinal injection. All of the above technical errors are due to misplaced cannula. In general these distortions can be easily recognized and the data can be rejected on those grounds.

Small aggregates of microspheres may not be recognized and can only be prevented by adequate mixing of the microsphere suspension immediately before injecting. Routine microscopic examination, size determination and rejection of batches with more than small amounts of bizarre shapes is called for. Separation and counting radioactivity in the suspending medium for signs of leaching of the radiolabel, and checking for contamination by other isotopes is also required.

Kaihara et al (1968) found that the lungs extracted 100 % of 15 and 50 μm in diameter microspheres injected into the venous system of anesthetized dogs. He proposed that the lung fraction could serve as an indicator of systemic arterio-venous shunts. Abdominal aortic injection with 15 ± 5 μm microspheres resulted in 10 % passing into the pulmonary bed while peripheral trapping of

larger microspheres was complete. In another series of experiments, left atrial injections of 15 um microspheres produced no difference in distribution when compared with 50 and 80 µm microspheres. This dichotomy may be due to the site of injection, the anesthetic used, or different range of sizes in the microsphere batches.

Hales (1973) found complete trapping of microspheres in the lungs of conscious sheep, in both control and heat stressed animals. Hales (1974) states that if 15 µm in diameter microspheres are used, capillary or nutrient flow is measured. If larger sizes are used, nutrient plus some portion of AV anastomotic (shunt) flow is measured. Microspheres of greater then 150 µm diameter are needed to reliably measure total anatomical organ flow. This seems impracticable due to the hemodynamic effects of many large microspheres. Hales also points out the importance of a thermoneutral environment for baseline determinations, especially when head flows are measured.

Foster and Frydman (1978) also found near complete lung extraction of 15 µm microspheres, in control and norepinephrine treated rats. Significant increases in the lung fraction point to the opening of arterio-venous shunts in the systemic circulation, though it does not indicate where these openings occur. Madden et al (1968) showed that rat liver microvascular beds have a smaller limiting vascular diameter than the lungs. Archie et al (1973) found virtually complete extraction of 15 µm spheres in dog

kidneys. Warren and Ledingham (1974) found no shunting of microspheres of 15, 25 or 50 µm diameter through the conscious rabbit gut, lung, liver or kidneys. However the number of microspheres in anesthetized rabbit lungs doubled over the conscious controls with 15 and 25 µm spheres. More then 80 % of spheres travelling to the ear went right through. Shunting in the rabbit hind limb increased from 7 % with 50 µm spheres to 18 % with 15 µm spheres.

Marcus et al (1976) examining regional cerebral blood flow found that less then 2 % of 15 µm microspheres passed through cerebral arterio-venous shunts in anesthetized dogs. Hypoxia and hypercapnia did not alter this value. The regional distribution was reproducible with calculated flows within 6 % of the mean.

Thus, from the above, using 15 µm spheres suspended in saline at normal room temperature, clearance of the blood by all major organs of microspheres should be near complete in the rat.

4 Microsphere Stability: Kaihara et al (1968) injected 50 µm microspheres and using external detectors monitored the radioactivity emanating from the heart and kidney regions. Allowing for radioactive decay, there was no change in organ radioactivity over two weeks. No trace of the isotope (46-Sc) was found in the urine and the feces. They concluded that carbonized microspheres were not metabolized and stayed in the capillaries almost

indefinitely.

Madden et al (1970) recovered "large numbers" after 15 µm microsphere injection in washings from isolated oesophagus, trachea and bronchial segments after right atrial injection in rats. The work suggests that microspheres may indeed migrate to some extent. The otherwise highly reproducible nature of this technique does not support this suggestion. Madden's recovered spheres had mean diameters of 6.3 to 7.2 µm. This indicates that the stated variation of 15 ± 5 µm microspheres may have been "somewhat" underestimated.

5 Minimum Number: Buckberg et al (1971) using statistical theory determined that in dogs, at least 400 microspheres per organ measured are necessary to ensure that the upper 95 % confidence limit be within 10 % of the actual flow. Errors due to random variation would be unacceptably high if smaller numbers are allowed. They found, in lambs and dogs, agreement between simultaneously withdrawn reference samples fulfilling this criterion, most points falling below the upper confidence limit. They attributed the slightly higher than predicted number that fell above the limit to non-random processes, presumably inadequate mixing. Buckberg and associates pointed out that one of the most important determinants of accuracy of the microsphere method is the number of microspheres per organ or sample, not the amount of radioactivity per sample as had been

assumed by some previous investigators.

Ishise et al (1980) found good correlation between cardiac output measured by the microsphere method and aortic flow measured by electromagnetic flowmeter in rats, using reference samples with more than just 200 microspheres per sample. Thus the minimum necessary number of microspheres per sample may in fact be quite flexible, from 200 to 400 microspheres, to reach a given level of accuracy.

6 Determining Amount Injected: For determination of the fractional distribution of blood flow, the total number of injected microspheres (injected radioactivity) must be known (Sasaki and Wagner 1971; Warren and Ledingham 1974; McDevett and Nies 1976; Morfaux et Bralet 1977). The above investigators measured syringe and connecting tube radioactivity before and after injection. Allowance must be made for counting geometry and radioactive decay. In the case of small animal studies the whole animal can be counted in the radiation measuring device, thereby making the amount of microspheres remaining in the injecting apparatus irrelevant (Mendell and Hollenberg 1971; Rakusan and Blahitka 1974; Stulcova 1977). Since all measurements in my study are made at approximately the same time, allowance for radioactive decay during the experiment is usually not necessary.

7 Reproducibility: Even with the minimum number

criterion satisfied, another possible source of error can arise. The microsphere method can measure not only the distribution of cardiac output but also cardiac output itself. This is done using an arterial reference sample withdrawn at a known rate during injection of the microspheres. Moore and associates (1981) found that the rate of withdrawal of the reference sample can affect accuracy of the cardiac output determination. A reference sample withdrawn at too low a rate can lead to falsely high cardiac output estimations. With reference samples containing over 400 microspheres, Moore et al found that a withdrawal rate of 0.65 % of the actual cardiac output gave better estimates of renal blood flow (and presumably cardiac output) then a withdrawal rate of 0.25 % in anesthetized dogs, 4.41 ± 1 ml/min·g and 6.92 ml/min·g, respectively. The former value agreed closely with values reported in the literature using other methods. The precise relationship between withdrawal rate and accuracy of the reference sample method has yet to be worked out.

All of the above conditions have been considered and satisfied for the experimental data reported in the results section of this thesis.

1.5

Statement of Objectives

This study was designed with four objectives in mind. The first objective was to measure cardiac output and

organ blood flows in senescent rats in comparison with younger rats.

The second objective of this study was to describe cardiovascular responses of the old rat to a stress and to contrast these responses to those in younger rats. Age related cardiovascular responses to stress are largely unknown and some studies on age in the past have used physically or mentally stressed animals. The conclusions of these authors may have been influenced by the variable of stress.

The third objective was to determine if age related changes in cardiovascular responses to anesthesia exist. If age related changes are found, anesthesia may have influenced the conclusions of earlier investigators.

The final objective of this study was to measure blood flow of the myocardium at different times after transplantation.

1.6

Experimental Design

The reader may have noticed that the above discussion was concerned almost entirely with the effects of ageing in the laboratory rat. Dogs, other animals, primates and Man have been used in the past but most age related work has been done on the rat for three reasons.

It has been shown that properly maintained aged rat colonies are almost entirely free of large vessel

arteriosclerosis and valvular heart disease (Simmes and Berg 1957; Weisfeldt et al 1971b). Virgin rats do not develop arteriosclerosis or coronary artery lesions in senescence (Wexler 1964 a,b) although myocarditis in three months old as well as fibrotic changes in twelve month old F-344 male rats can be seen (Roberts and Goldberg 1979). Using the rat for this study minimized the complicating factors of disease.

Secondly, the maximum lifetime of a rat is almost three years (Zivic Miller personal communication). This avoids expensive, unwieldy and long study periods.

Third, multi-organ blood flow measurements can not easily and accurately be performed on human subjects. The use of an animal experimental model avoids this difficulty.

Sprague Dawley rats of five months of age were chosen to make up a "young adult" group because adolescent growth has stopped at this time and changes could be designated as ageing rather then developmental. Twenty four months old SD rats were chosen as the "senescent" group because by this time at least one half of the cohort has died (Schlettwein-Gsell 1970). Twelve months old SD rats were arbitrarily chosen as an intermediate "middle age" group.

In addition to the basal data on regional blood flows in three age groups (control data), the response to a stress in the same rat was also obtained for the second objective of this study. Isoproterenol infusion was decided

upon, as sympathetic activation is a common denominator in different types of stress, and the response of beta adrenergic stimulation in particular has been shown to change with age.

The third objective is an investigation of the effect of pentobarbital anesthesia on blood flow distribution in Lewis rats at five and ten months of age. Comparison with unanesthetized blood flow distribution in the same rat (control) was made.

Myocardial blood flow in the perfused heterotopic isotransplanted rat heart (Tolnai and Korecky 1980) has not yet been adequatly investigated. Any change with age of the blood flow in this denervated preparation is therefore unknown. The fourth objective of this study is the measurement of blood flow in this denervated heart. The studies were made in the same five and ten months old Lewis rats used above, each having had its transplant installed four weeks or seven months earlier, respectively.

In summary, cardiac output and its distribution in senescent laboratory rats were compared to younger rats. In the same animals, cardiovascular responses to an isoproterenol stress were also examined. A different strain of rat was investigated to confirm any age changes in the cardiac output and its distribution, with special emphasis on the transplanted hearts carried in these animals. The response to pentobarbital anesthesia at two different ages was also measured in this second group.

Chapter 2

Methods

2.1

The Effects of Age and Isoproterenol

As described in the introduction, experiments were performed on two strains of rats. Control cardiac output distributions and those obtained after isoproterenol infusion were determined in three age groups of Sprague Dawley rats. Control cardiac output distributions and those obtained after pentobarbital administration were determined in two age groups of Lewis f/Mai rats which had previously been heterotopically isotransplanted with a donor rat heart in the abdomen.

2.1.1 Animals: Male Sprague Dawley rats were obtained from Zivic-Miller and from Harlen Industries. At the time of the experiment the rats were five, twelve and twenty four months of age and weighed 641 ± 69, 690 ± 130 and 733 ± 108 grams respectively. The rats were kept in a twelve hour light/twelve hour dark cycle at twenty two degrees celsius and a relative humidity of fifty percent. Purina Rat Chow 5012 and fresh water were continuously available. All animals were acclimatized to our animal house for at least one week, usually longer.

2.1.2 Surgical Procedure: The rats were allowed only

water for eighteen to twenty four hours prior to the operation. Initially sodium pentobarbital 45 mg/kg IP was used to anesthetize the rats. After numerous anesthetic deaths at doses as low as 20 mg/kg, innovar (Innovar-Vet) 0.03 ml/100 g IM was substituted in the two older age groups and gave satisfactory anesthesia. Out of the same shipment of 1 and 2 years old rats, 7 out of 7 rats injected with nembutal died, but only 1 out of 6 rats injected with Innovar-Vet died.

It was felt that left atrial cannulation via thoracotomy (Wicker and Tarazi 1982) would be accompanied by an extremely high mortality rate in the older groups. Accordingly, each rat was cannulated using the method of Malik et al (1976) and McDevitt and Nies (1976). Briefly, the right carotid artery was cannulated with PE-10 tubing. A metal stylet was inserted and the cannula advanced until a change of resistance was felt, indicating entry into the left ventricle. This was accompanied by arrhythmias and QRS complex changes on the ECG which reverted to normal when the metal stylet was removed. Correct position was ensured by removing the stylet and monitoring the pressure with a Statham P23Gb pressure transducer connected to a Grass Model 7 Polygraph. The position was confirmed at necropsy.

The abdominal aorta was cannulated with PE-10 tubing through the left femoral artery. Extravascular PE-50 extensions of the ventricular and aortic cannulae were passed under the skin and externalized at the back of the

neck. All incisions were closed with 4/0 silk and the cannulae refilled with heparinized saline and flame sealed. The animals were left to recover overnight.

2.1.3 Injection of Microspheres: On the day of the experiment each animal was placed in a plastic restraining cage (Rakusan and Blahitka 1974). The cannulae were flushed with heparinized saline and connected to the pressure monitoring device to confirm proper placement and patency and to determine the heart rate.

The vial containing the microspheres was agitated for one minute on a vortex mixer immediately before injection. After heparinizing the rat, approximately 90,000 (2 μCi) of either 141-Ce or 51-Cr labeled 15 + 3 μm carbonized microspheres, (10 mCi/g, New England Nuclear), suspended in 0.05 ml saline and 0.01 % Tween 80, were drawn into a 1 ml tuberculin syringe and injected through the ventricular cannula. Simultaneously blood was withdrawn at a rate of 0.79 ml/min (between 0.4 and 0.6 % of expected cardiac output) from the aortic cannula with a Harvard Apparatus Co. infusion / withdrawal syringe pump. The volume withdrawn, about 1 ml, was replaced with heparinized saline used to flush first the microspheres and then the blood remaining in the reference cannula into the animal. This is the control determination of the distribution of cardiac output.

2.1.4 Infusion of Isoproterenol: Each animal was then infused with l-isoproterenol HCl dissolved in saline at 0.2 µg/min·kg at a rate of 0.19 ml/min through the cardiac cannula for three to five minutes. At the end of this period the heart rate was recorded again with a pressure tracing monitored through the aortic cannula. Phasic blood pressure could not be determined due to severe damping of the pressure wave form because of the length and small diameter of the cannula. The withdrawal pump was reattached and the complementary microsphere suspension was injected as before. This is the determination of cardiac output after isoproterenol infusion. The animal was then killed with an overdose of sodium pentobarbitol or saturated KCl through the cardiac cannula.

2.1.5 Preparation of Tissue: The organs were immediately removed and weighed. The cerebral hemispheres, cerebellum and brainstem were taken together, the left ventricle of the heart and the free wall of the right ventricle were separated. The intestinal contents were gently expressed by hand and discarded. The mesentery, omentum and pancreas were left with the carcass. As a sample of skeletal muscle tissue, the right gastrocnemius was removed. All of the skin (with hair) except that over the tail, paws and face was removed from the carcass and weighed. The carcass portion includes all organs and tissues not otherwise mentioned in the tables (see Results

section). Small organs were dissolved in 10N KOH in plastic test tubes. The liver, skin, carcass and sometimes the intestine were dissolved in beakers containing 10N KOH after which two well mixed 5 ml aliquots of each were placed in plastic test tubes and measured.

2.1.6 Measurement of Radiation and Calculations: The radioactivity of all tissues was counted for at least 50 minutes in a two channel well type Packard gamma counter. Each channel was set to bracket the primary gamma energy peak of one of the two isotopes used. For all tissues, the count in each channel was converted into either the control fraction of cardiac output or the fraction of cardiac output after isoproterenol infusion, allowing for overlap of one channel on the other (after Rudolph and Heymann 1967; and Hales 1974). The following formulae were used for calculation of the results:

Total Count=sum of Organ Counts+Reference Count

Fraction of Cardiac Output=Organ Count/Total Count

Cardiac Output=(Total Count X k)/Reference Count

Organ Blood Flow=Fraction of Cardiac Output X Cardiac Output

(note that k = reference sample withdrawal rate.
ie. k = 0.79 ml/min).

The data were analysed with the aid of an Apple][Plus computer and a Wang 600 programmable calculator. Standard statistical methods were used to calculate descriptive

statistics (Snedecor and Cochran 1967). Paired t tests were performed in each age group between control and experimental data. One way ANOVA was used to compare age groups, once for each control value and again for experimental values. If the ANOVA indicated significant differences, Tukey's method for simultaneous multiple comparisons (Wallenstein and co-workers 1980) was used to detect where the difference was located. Linear regression was used to detect trends between age groups.

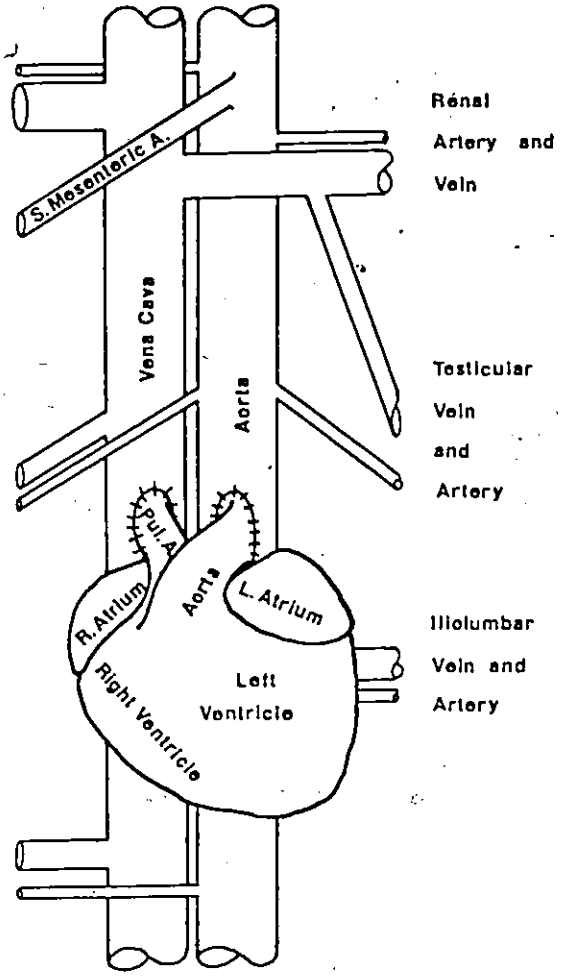
2.2

Effect of Age and Pentobarbital

2.2.1 Animals: Inbread male Lewis f/Mai rats at five and ten months of age weighing 347 ± 20 and 476 ± 23 grams respectively, were obtained. Originally from Microbiological Associates (Bethesda, Maryland), they had been transplanted in our lab with a heart from a similar rat four weeks or seven months previously (Tolnai and Korecky 1980) by an experienced technician. All rats were 3-4 months old at the time of this procedure. In this preparation abdominal aortic blood from the recipient enters the transplant vasculature through the coronary ostia, perfuses the beating heart normally and exits via the coronary sinuses and Thebesian veins in the right atrium. It is then pumped into the recipient vena cava. The transplant right ventricle therefore, pumps only the blood

that perfuses the transplant. The left ventricle is empty. Each transplanted heart was beating vigorously (190 ± 56 and 209 ± 87 BPM, respectively). (see figure 1).

Each rat was treated according to the following procedure.



SCHEMATIC OF LOCATION OF TRANSPLANTED RAT HEART IN ABDOMEN

Figure 1. A transplanted heart of the same age as the recipient is retrogradely perfused via the coronary arteries and drained through the pulmonary artery. This is a stable, long term, in vivo model of cardiac atrophy that before now has not been investigated with the microsphere technique.

2.2.2 Experimental Procedure: The rats were allowed only water for eighteen to twenty four hours prior to surgery. Sodium pentobarbital 40-45 mg/kg IP was used. Heart rates in the transplanted hearts were taken at this time using bipolar leads positioned on either side of the abdomen. The two age groups were cannulated as in the previous description. All incisions were closed, at which point the unconscious rat was placed in the prone position characteristic of awake rats (normal rat posture) and left for at least two minutes. Then 141-Ce labeled microspheres were injected as described in the preceding section. This is the experimental (pentobarbital treated) determination of cardiac output distribution. During this time the rat was still surgically anesthetized.

Each rat was allowed to recover overnight with access to water only. One day after the cannulation a second injection of microspheres labeled with 51-Cr was given to each conscious rat. This is the control determination of the distribution of cardiac output.

Tissue preparation, radiation counting and calculations were similar to the description in Method 1.

Chapter 3

Results

3.1

Effects of Age and Isoproterenol

Considerable thought has been given to arriving at the best format to present the results of these experiments. For each age group of the two strains of rat, "control" values of each organ's fraction of the cardiac output as well as "control" values for blood flow per unit of organ mass have to be presented. Then the effect of either isoproterenol or pentobarbital on the two parameters needs to be presented.

Comparisons must be made of control values in each organ of each age in the two strains, as well as with the experimental effects in each organ and each age group. Incidental information such as heart rate, stroke volume, organ weights and others must be presented too.

In order to do all of this, liberal use has been made of tables. Significant differences (by ANOVA) and trends (by linear regression) have been noted by symbols as described in the legend at the bottom of each table.

Results will be presented in two sections, the first dealing with the control and isoproterenol treated SD rats, the second dealing with control and pentobarbital treated Lewis rats. The primary interest of this study, the

first stated objective in the introductory chapter, is the characterization of the distribution of cardiac output in the undisturbed senescent rat. This can be properly evaluated only when compared with younger animals. The first three tables therefore compare the control data of the three different age groups of SD rats; 1) general cardiovascular parameters, 2) each organs fraction of the total cardiac output and 3) each organs blood flow per unit of organ mass. The next three tables deal, in the same order as the first three, with cardiovascular effects of isoproterenol.

The second section deals with the two age groups of Lewis rats. The first three tables compare the control data of general cardiovascular parameters, each organs fraction of the total cardiac output, and the organ blood flow per unit of organ mass. The next three tables deal, in the same order, with cardiovascular effects of pentobarbital.

Additional data on organ weights, organ fractions of cardiac output and organ blood flows after isoproterenol or pentobarbital administration are included in the appendix.

3.1.1

Effects of Age on General Cardiovascular Parameters in Normal Sprague Dawley Rats

The primary intention of this thesis is to

investigate what happens in the normal circulatory system as an animal gets older. More specifically, what happens to the amount and distribution of blood leaving the heart and to various organ blood flows, in the unanesthetized male rat at 5, 12 and 24 months of age. This corresponds to young adult, middle aged and old rats. Distribution of blood flow is expressed here as a collection of percentages of the whole cardiac output. Although not usually directly comparable, in this thesis comparisons were made because the absolute cardiac outputs were all quantitatively similar and not statistically different. (See Table 1). Total body weights were similar as well, so cardiac output per kilogram of body weight was not significantly different in the three age groups. Heart rates as measured from ECG tracings were found also to closely correspond, and therefore calculated stroke volumes were not significantly different. Any differences in the fraction of cardiac output going to an organ in rats of different ages will therefore reflect one of three things: either a change in the weight of an organ, a difference in the actual tissue perfusion, or a combination of the two. Differences will be referred to as "age related" or "affected by age" in reporting the results obtained. It should be understood that factors other than age and organ weights are also involved. For example, an infectious disease may wipe out most of a colony of "aged" rats. The remaining rats would be a selected subset of, and not necessarily representative of, all "aged rats." An

investigator studying this group might be misled into believing that differences uncovered between these rats and a group of young adult rats are solely the result of ageing. The choice of a reputable supplier of the experimental animals avoids many difficulties of this type.

The results are summarized in tables and figures immediately following each section. Statistical significance is at the 5 % level unless otherwise stated.

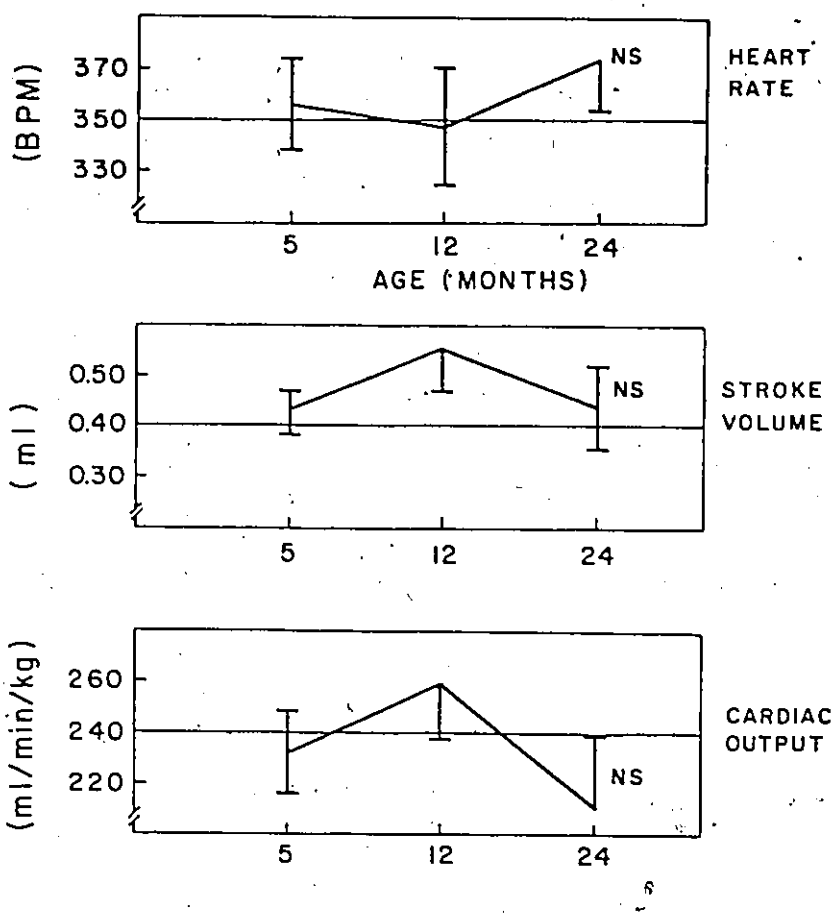


Figure 2. Heart rate, cardiac output and stroke volume from three age groups of SD rat. No significant age related differences were found. Presented as mean ± S.E. n = 7 in each age group.

The results were gathered from twenty-one male SD rats of three different ages. No age related differences were found in these groups as is shown in Table 1 and Fig 2.

Table 1
General Cardiovascular Parameters
Effect of Age

Age group:	5 months n=7	12 months n=7	24 months n=7
Heart Rate (BPM)	356 ± 18	348 ± 23	374 ± 20
Stroke Volume (ml)	0.43 ± 0.04	0.55 ± 0.08	0.44 ± 0.08
Mean Arterial Pressure (mm-Hg)	113 ± 2	93 ± 5	92 ± 11
Cardiac Output (ml/min)	150 ± 16	182 ± 25	158 ± 27
Cardiac Output/kg (ml/min·kg)	232 ± 16	259 ± 22	211 ± 28
Cardiac Output/M~2 (ml/min·M~2)	2201 ± 175	2508 ± 251	2105 ± 307

All values are ± standard error. No statistically significant differences (by ANOVA) or trends (by linear regression) were found in these values.

The distribution of cardiac output was found to be affected by age (see Table 2 and Fig 3). The splenic and renal fractions of cardiac output decreased with age ($p<0.05$ ANOVA, $p<0.01$ linear regression), the differences between the youngest and the oldest groups being significant (Tukey's method). The cerebral fraction of cardiac output

increased significantly from 5 to 12 months and then fell from the 12 to the 24 months old animals. The hepatic arterial and gastric fractions of cardiac output increased significantly from those found in the five months old animals to a high value in the twelve months old animals, and then appeared to fall again to values comparable to those in the first group ($p<0.05$ ANOVA). No other significant differences were found.

Table 2
Distribution of Cardiac Output
% of C.O. in Three Age Groups

Organ	5 months n=7	12 months n=7	24 months n=7	
Right Ventricle	0.69 ± 0.11	1.04 ± 0.20	0.77 ± 0.03	
Left Ventricle	4.04 ± 0.39	5.23 ± 1.35	3.97 ± 0.15	
Total Heart	4.71 ± 0.42	6.27 ± 1.53	4.73 ± 0.15	
Lungs	1.36 ± 0.17	2.15 ± 0.84	1.64 ± 0.11	
Diaphragm	0.53 ± 0.09	0.57 ± 0.08	0.67 ± 0.10	
Liver Art.	1.66 ± 0.25	4.15 ± 0.93	2.20 ± 0.65	@
Spleen	1.92 ± 0.14	1.56 ± 0.22	1.12 ± 0.18	@ ##
Stomach	0.91 ± 0.13	1.52 ± 0.23	1.08 ± 0.11	@
Small Intestine	7.42 ± 0.64	8.36 ± 1.45	7.21 ± 1.07	
Large Intestine	2.80 ± 0.63	2.20 ± 0.56	2.51 ± 0.34	
Portal	13.05 ± 1.24	13.63 ± 1.66	11.27 ± 1.46	
Kidneys	18.19 ± 2.25	16.28 ± 0.94	10.71 ± 1.46	@ ##
Adrenals	0.15 ± 0.03	0.15 ± 0.03	0.24 ± 0.05	
Testes	0.41 ± 0.07	0.52 ± 0.04	0.38 ± 0.04	
Brain	1.19 ± 0.08	1.76 ± 0.25	1.17 ± 0.09	@
Gastrocnemius	0.41 ± 0.08	0.23 ± 0.05	0.35 ± 0.06	
Skin	12.20 ± 1.07	14.71 ± 1.86	16.43 ± 1.74	
Carcass	45.61 ± 3.84	39.21 ± 2.26	48.87 ± 3.21	

"@" means ($p<.05$) in one way ANOVA.
"##" means ($p<.01$) in linear regression. Expressed as % of C.O. ± standard error. Lung refers to bronchial fraction and A-V shunts. Liver Art. refers to hepatic artery fraction. Portal refers to combined Spleen, Stomach, Small and Large Intestine fractions. Carcass is the remaining tissue, mostly bone, muscle and fat.

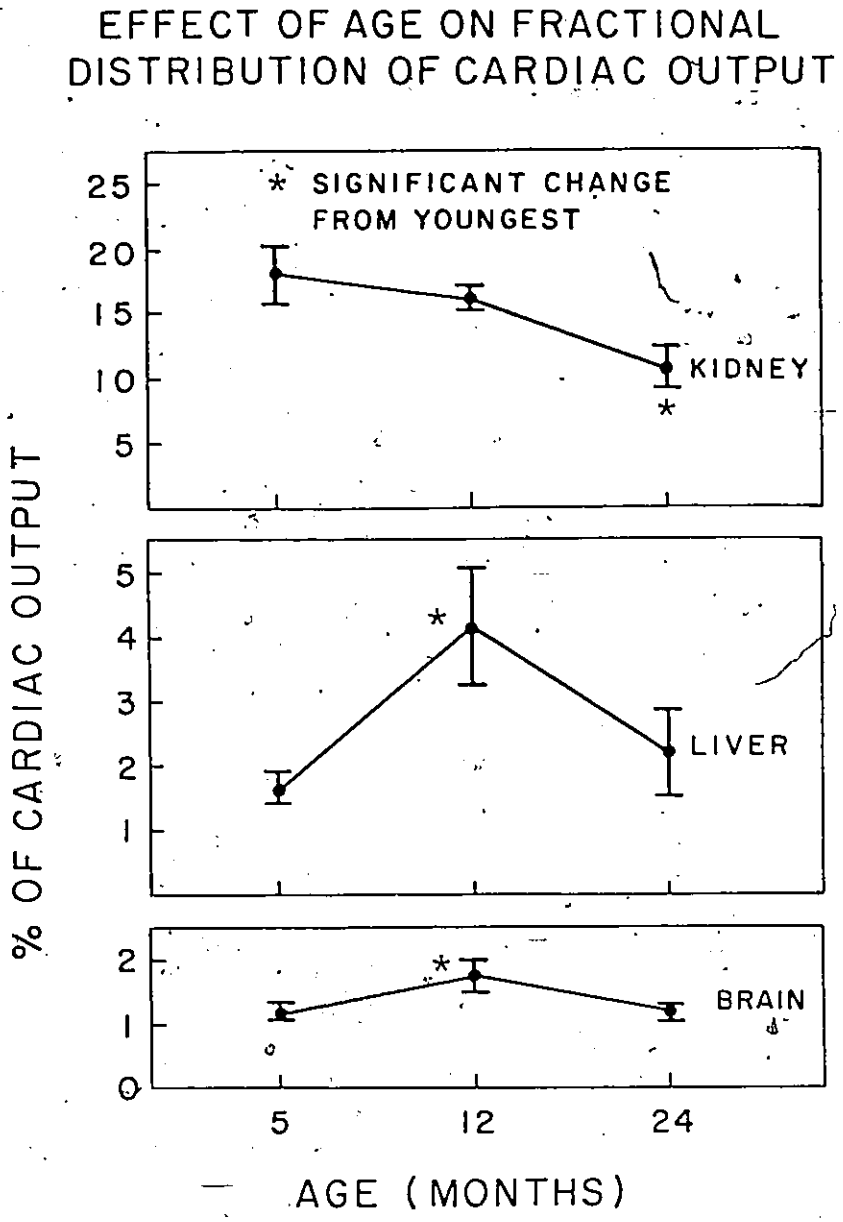


Figure 3. Examples of age related changes in the fraction of cardiac output to different organs. The value of the kidney fraction falls from youngest to oldest. The spleen also showed this trend. The arterial liver, the brain and stomach fractions increase from the youngest to the middle age group and appear to fall in the oldes group. Other organ fractions were found not to change. Presented as mean ± S.E. n = 7 in each age group.

The blood flows in terms of blood flow per unit of wet organ weight also varied with age in some organs (see Table 3). The flow per gram of renal tissue fell with age from the youngest to the oldest group ($p<0.05$ ANOVA, $p<0.01$ linear regression), the difference between the youngest and oldest group being significant. The small intestinal blood flow and the portal blood flow appeared to rise from the values in the five months old rats to a high in the twelve months old animals and then significantly fell in the twenty four months old rats to levels comparable to the first group. Several other organs exhibited this rising and falling trend but differences were not statistically significant, ie. the liver and the brain.

Table 3
Organ Blood Flows
As ml/min·g in Three Age Groups

Organ	5 months n=7	12 months n=7	24 months n=7	
Right Ventricle	3.16 ± 0.52	5.53 ± 1.03	3.84 ± 0.59	
Left Ventricle	4.60 ± 0.52	6.31 ± 1.17	4.48 ± 0.67	
Total Heart	4.32 ± 0.47	6.14 ± 1.12	4.33 ± 0.63	
Lungs	0.78 ± 0.18	0.98 ± 0.41	0.63 ± 0.12	
Diaphragm	0.51 ± 0.11	0.60 ± 0.12	0.65 ± 0.12	
Liver Art.	0.12 ± 0.02	0.29 ± 0.03	0.15 ± 0.05	
Spleen	2.48 ± 0.26	2.84 ± 0.66	1.37 ± 0.28	
Stomach	0.64 ± 0.11	0.96 ± 0.20	0.49 ± 0.07	
Small Intestine	1.40 ± 0.11	2.58 ± 0.58	1.32 ± 0.11	@
Large Intestine	1.06 ± 0.30	1.09 ± 0.34	1.00 ± 0.19	
Portal	1.20 ± 0.11	1.83 ± 0.33	1.07 ± 0.09	@
Kidneys	5.81 ± 0.74	5.17 ± 0.58	2.93 ± 0.54	@ ##
Adrenals	2.10 ± 0.34	2.63 ± 0.48	3.36 ± 0.52	
Testes	0.15 ± 0.03	0.23 ± 0.03	0.16 ± 0.02	
Brain	0.84 ± 0.11	1.26 ± 0.20	0.77 ± 0.14	
Gastrocnemius	0.19 ± 0.04	0.13 ± 0.04	0.22 ± 0.05	
Skin	0.12 ± 0.02	0.15 ± 0.03	0.13 ± 0.03	
Carcass	0.16 ± 0.02	0.15 ± 0.02	0.16 ± 0.03	

"@" means ($p<.05$) in one way ANOVA.
"##" means ($p<.01$) in linear regression. Expressed as ml/min·g ± standard error. Lung refers to bronchial flow and A-V shunts. Liver Art. refers to hepatic artery flow. Portal refers to combined Spleen, Stomach, Small and Large Intestine flows. Carcass is remaining tissue, mostly bone, muscle and fat.

3.1.2

Effects of Isoproterenol at Different Ages

Control data and, in the same animals, data obtained after isoproterenol infusion were obtained in each age group. Results were evaluated by using the paired t-test.

The infusion of 0.2 µg/min·kg of l-isoproterenol HCl via a carotid artery into the left ventricle for three to five minutes produced rather dramatic increases over control values in heart rate ($p<0.01$ t-test), cardiac output, cardiac output per kilogram and cardiac output per square meter ($p<0.05$ t-test) in the five months old group. (see Table 4 and Fig 4). Heart rate increased over controls in the two older groups ($p<0.01$) as well, but the response of the heart rate to isoproterenol decreased with age ($p<0.05$ linear regression) in the two older age groups. Thus no significant differences in the cardiac outputs were found in these older groups.

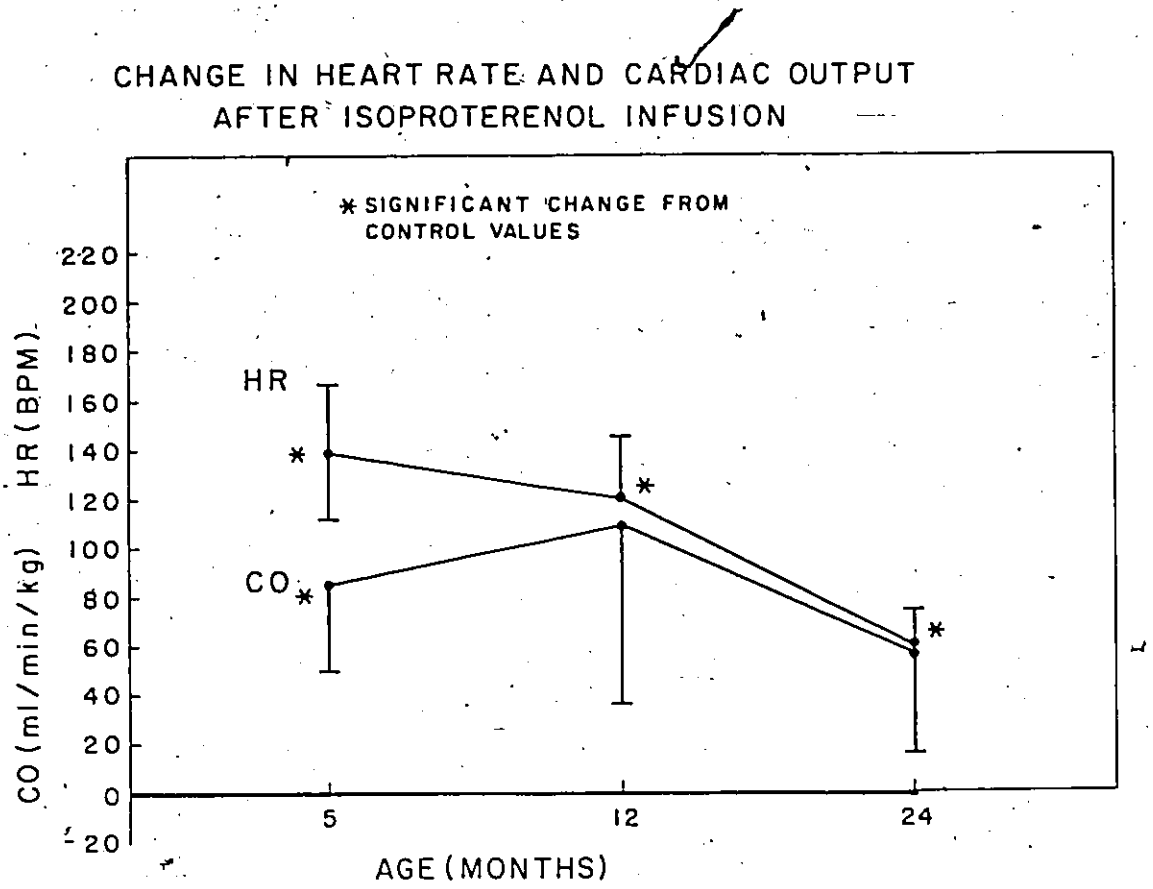


Figure 4. The increases in heart rate and cardiac output over control values after infusion of isoproterenol at a rate of 0.2 μg/kg·min for 3-5 minutes. Heart rate is significantly higher in all age groups while cardiac output increases significantly only in the youngest group. Presented as mean ± S.E. n = 7 in each group.

Table 4
General Cardiovascular Parameters
Effect of Age and Isoproterenol Infusion

Age group:	5 months n=7	12 months n=7	24 months n=7
Heart Rate (BPM)			
Cont.	356 ± 18	348 ± 23	374 ± 20
Isop.	494 ± 22 **	469 ± 13 **	435 ± 23 ** #
Diff.	139 ± 27	121 ± 24	61 ± 14
Stroke Volume (ml)			
Cont.	0.43 ± 0.04	0.55 ± 0.08	0.44 ± 0.08
Isop.	0.41 ± 0.03	0.53 ± 0.08	0.49 ± 0.15
Diff.	-0.03 ± 0.07	-0.02 ± 0.13	0.06 ± 0.10
Mean Arterial Pressure (mm-Hg)			
Cont.	113 ± 2	93 ± 14	92 ± 11
Isop.	113 ± 5	84 ± 30	77 ± 10
Diff.	0 ± 4	-10 ± 7	-15 ± 7
Cardiac Output (ml/min)			
Cont.	150 ± 16	182 ± 25	158 ± 27
Isop.	200 ± 15 *	247 ± 40	199 ± 48
Diff.	50 ± 19	66 ± 47	41 ± 29
Cardiac Output/kg (ml/min·kg)			
Cont.	232 ± 16	259 ± 22	211 ± 28
Isop.	316 ± 30 *	366 ± 60	267 ± 56
Diff.	84 ± 34	109 ± 72	56 ± 39
Cardiac Output/M~2 (ml/min·M~2)			
Cont.	2201 ± 175	2508 ± 251	2105 ± 307
Isop.	2978 ± 262 *	3517 ± 562	2657 ± 587
Diff.	777 ± 308	1009 ± 705	553 ± 387

"*" means ($p<0.05$), "**" means ($p<0.01$) in paired t-test with controls after 3 min. infusion of 0.2 µg/kg·min Isoproterenol HCl (Isop.).
"#" means ($p<0.05$) in linear regression between age groups.
Diff. means difference in mean values. All values are ± standard error. No statistically significant differences (by ANOVA) or trends (by linear regression) were found in the controls (Cont.).

In the five months old rat group only the fraction of cardiac output to the right ventricle increased significantly over control values after isoproterenol (see Table 5). In the twelve months old rat group the spleen, stomach, small intestine, kidney, testes and portal fractions all were significantly less than control values after infusion of isoproterenol. In the twenty four months old rat group only the spleen and the portal fractions of cardiac output changed significantly, also decreasing. The fraction of cardiac output to the liver and skin, after isoproterenol infusion, increased with age ($p<0.05$ and $p<0.01$, respectively, in linear regression) in the groups studied, the difference between the youngest and the oldest group being significant. The fraction to the spleen decreased ($p<0.05$ linear regression) with age, both the middle age and senescent groups being significantly lower than the youngest age group.

Table 5
Change In Fraction of Cardiac Output
After Isoproterenol Infusion
In Three Age Groups
as a Percentage of the Control

Organ	5 months n=7	12 months n=7	24 months n=7
Right Ventricle	(+) 65 ± 23 *	(+) 55 ± 19	(+) 1 ± 18
Left Ventricle	(+) 52 ± 23	(+) 36 ± 26	(-) 20 ± 18 #
Total Heart	(+) 54 ± 23	(+) 39 ± 24	(-) 17 ± 16 #
Lungs	(+) 6 ± 31	(+) 19 ± 41	(-) 9 ± 21
Diaphragm	(-) 10 ± 19	(-) 9 ± 16	(+) 5 ± 17
Liver Art.	(-) 13 ± 10	(+) 15 ± 14	(+) 192 ± 94 #
Spleen	(-) 16 ± 15	(-) 46 ± 8 **	(-) 26 ± 9 *
Stomach	(+) 39 ± 57	(-) 39 ± 11 *	(-) 34 ± 14
Small Intestine	(-) 22 ± 9	(-) 47 ± 11 **	(-) 30 ± 12
Large Intestine	(-) 3 ± 14	(+) 29 ± 67	(-) 32 ± 11
Portal	(-) 18 ± 9	(-) 43 ± 11 **	(-) 31 ± 10 *
Kidneys	(-) 17 ± 12	(-) 56 ± 10 **	(-) 35 ± 17
Adrenals	(+) 5 ± 11	(+) 40 ± 26	(-) 1 ± 21
Testes	(-) 1 ± 16	(-) 28 ± 8 *	(+) 19 ± 13
Brain	(+) 6 ± 12	(-) 34 ± 15	(-) 22 ± 17
Gastrocnemius	(+) 15 ± 31	(+) 149 ± 97	(+) 50 ± 38
Skin	(+) 1 ± 8	(+) 52 ± 34	(+) 63 ± 29
Carcass	(+) 15 ± 9	(+) 27 ± 9 *	(-) 8 ± 7

Change in fraction of cardiac output in each organ due to isoproterenol infusion ± standard error. The actual values are found in Table 15 of the Appendix.
(+) indicates increase from control values.
(-) indicates decrease from control values.
"*" means ($p<0.05$), "**" means ($p<0.01$) significance of change from control values.
"#" means ($p<0.05$) in linear regression.

Blood flows after isoproterenol infusion, normalized for weight, also changed in some organs. Blood flow to the two ventricles of the heart increased over controls in the youngest age group (see Table 6 and Fig 5). Skin and carcass flows also increased in this age group ($p<0.05$, t-test). In the twelve months old group, flow to the right ventricle and the gastrocnemius muscle increased

over control values. Renal and small intestinal blood flow decreased significantly ($p<0.01$ and $p<0.05$, respectively, in t-test. No significant changes from control values were found in the twenty four months old group. After isoproterenol infusion, blood flows to the spleen, stomach and kidneys were found to decrease with age ($p<0.01$ linear regression).

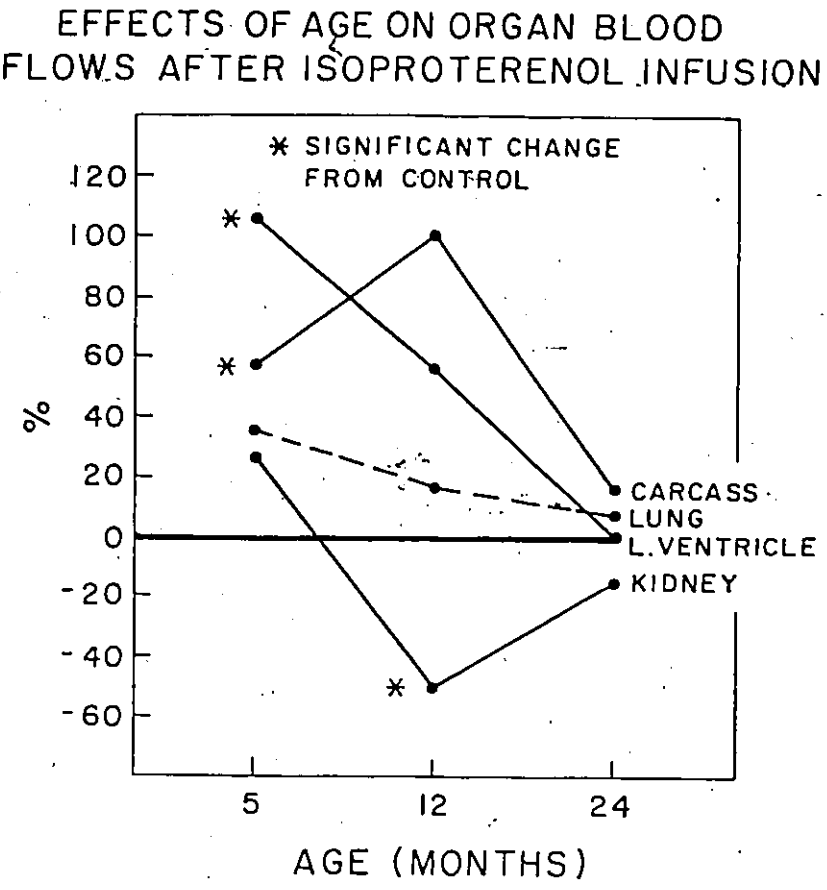


Figure 5. Example of age related changes in the blood flow to different organs after 3-5 minutes infusion of 0.2 μg/kg·min isoproterenol. Blood flow to the heart and carcass was most increased in the youngest age group. The renal blood flow fell significantly in only the middle age group. No blood flow changes were found in the oldest age group. Other organs such as the bronchi of the lungs did not exhibit blood flow changes due to isoproterenol. Presented as mean. n = 7 in each group.

Table 6
Change in Organ Blood Flow Per Gram
In Three Age Groups
After Isoproterenol Infusion
as a Percent of Control

Organ	5 months n=7	12 months n=7	24 months n=7
Right Ventricle	(+)119 ± 28 *	(+) 85 ± 14 *	(+) 24 ± 22 #
Left Ventricle	(+)106 ± 35 *	(+) 57 ± 30	(+) 1 ± 26 #
Total Heart	(+)107 ± 33 *	(+) 62 ± 26	(+) 4 ± 25 #
Lungs	(+) 36 ± 31	(+) 17 ± 31	(+) 8 ± 21
Diaphragm	(+) 25 ± 29	(+) 32 ± 26	(+) 7 ± 11
Liver Art.	(+) 26 ± 23	(+)106 ± 67	(+)252 ± 96 #
Spleen	(+) 19 ± 24	(-) 29 ± 11	(+) 0 ± 21
Stomach	(+) 77 ± 56	(-) 19 ± 15	(-) 12 ± 23
Small Intestine	(+) 14 ± 23	(-) 37 ± 9 *	(-) 13 ± 19
Large Intestine	(+) 37 ± 27	(+) 32 ± 42	(-) 17 ± 14
Portal	(+) 18 ± 23	(-) 31 ± 9	(-) 20 ± 19
Kidneys	(+) 26 ± 31	(-) 50 ± 12 **	(-) 15 ± 29
Adrenals	(+) 45 ± 22	(+)138 ± 89	(+) 24 ± 24
Testes	(+) 43 ± 31	(-) 1 ± 12	(+) 60 ± 34
Brain	(+) 40 ± 27	(-) 22 ± 15	(-) 8 ± 20
Gastrocnemius	(+) 49 ± 32	(+)197 ± 78 *	(+) 70 ± 28
Skin	(+) 38 ± 12 *	(+)202 ± 137	(+)110 ± 49
Carcass	(+) 58 ± 18 *	(+)101 ± 52	(+) 17 ± 15

Change in organ blood flows due to isoproterenol infusion ± standard error. The actual organ blood flows are found in Table 16 of the Appendix.
(+) indicates increase from control values.
(-) indicated decrease from control values.
"*" means ($p<0.05$), "**" means ($p<0.01$) significance of change from control value.
"#" means ($p<0.05$) in linear regression.

3.2

Effect of Age on Conscious Lewis Rats

Body weight in Lewis f/Mai rats was found to increase with age from the five months old to the ten months old animals used in this study. Heart rate, stroke volume, cardiac output or cardiac output per kilogram of body weight were found not to differ significantly among the controls (see Table 7 and figure 6).

The weight of the left ventricle increased with age in the recipient hearts of the Lewis rats. The transplanted hearts increased in weight with increasing duration of transplant and age, especially the right ventricle. The weights are summarized in the following table.

Heart Weight (in g)
For Recipient and Transplanted
Lewis f/Mai Rats

	5 Months old n=7	10 Months old n=6	
Recipient			
Right Ventricle	0.14 ± 0.01	0.14 ± 0.02	
Left Ventricle	0.65 ± 0.02	0.74 ± 0.02	*
Total Heart	0.79 ± 0.03	0.88 ± 0.04	
Transplant			
Right Ventricle	0.07 ± 0.02 (-50%)	0.20 ± 0.02 (+43%)	**
Left Ventricle	0.31 ± 0.03 (-52%)	0.47 ± 0.01 (-36%)	**
Total Heart	0.39 ± 0.04 (-51%)	0.66 ± 0.03 (-25%)	**

"*" means ($p<0.05$), "**" means ($p<0.001$) by t-test between age groups.
Expressed as mean ± standard error.
Numbers in brackets indicate % difference from corresponding recipient hearts.

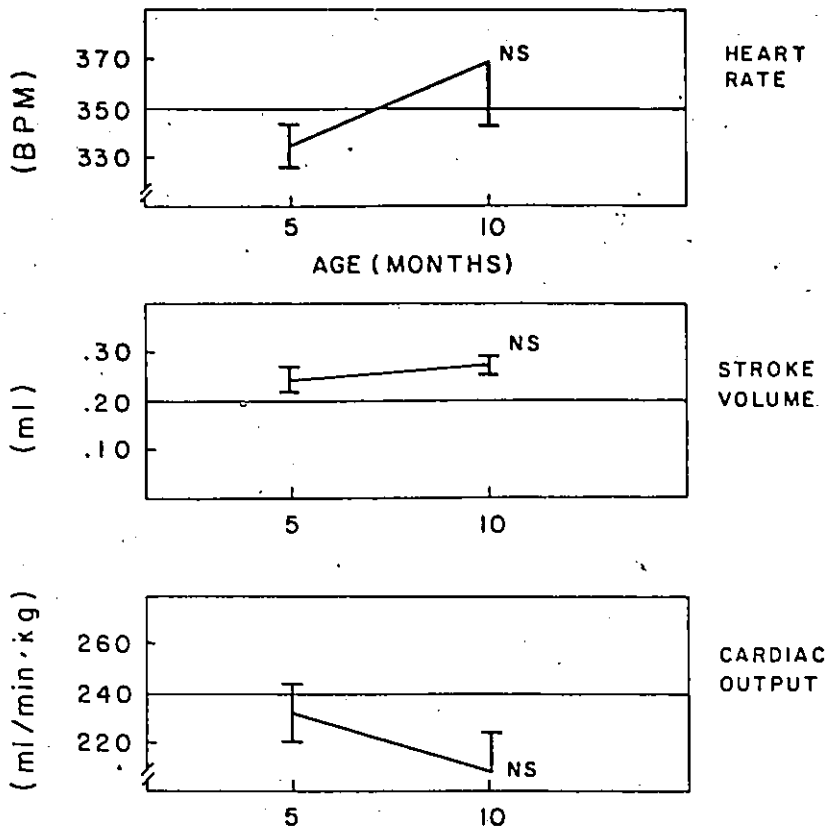


Figure 6. Heart rate, cardiac output and stroke volume from two age groups of Lewis rats. No significant age related differences were found. These results compare favorably with Figure 2. Presented as mean ± S.E. n = 7 in the younger group, n = 6 in the older age group.

Table 7
General Cardiovascular Parameters
Effect of Age on Lewis Rats

Age group:	5 months n=7	10 months n=6
Heart Rate (BPM)	334 ± 9	369 ± 27
Stroke Volume (ml)	0.24 ± 0.02	0.27 ± 0.02
Cardiac Output (ml/min)	81 ± 5	99 ± 8
Cardiac Output/kg (ml/min/kg)	232 ± 12	207 ± 16

All values are mean ± standard error in unanesthetized Lewis f/Mai rats. No statistically significant differences (by t-test) were found.

The fractions of cardiac output to the hepatic artery and the transplanted right ventricle increased in the ten month old animals compared to the five month old animals (see Table 8). The lung and the brain fractions were found to decrease with age ($p<0.05$ unpaired t-test between age groups).

Table 8.
Distribution of Cardiac Output
% of C.O. in Two Age Groups

Organ	5 months n=7	10 months n=6	
Right Ventricle	0.64 ± 0.11	0.52 ± 0.06	
Left Ventricle	3.66 ± 0.34	3.74 ± 0.38	
Total Heart	4.31 ± 0.43	4.26 ± 0.43	
Lungs	3.21 ± 0.60	1.15 ± 0.17	*
Liver Art.	0.94 ± 0.08	1.81 ± 0.37	*
Spleen	2.94 ± 0.48	2.18 ± 0.29	
Stomach	1.64 ± 0.10	1.74 ± 0.17	
Intestine	9.49 ± 0.57	8.68 ± 0.92	
Portal	11.13 ± 0.60	10.43 ± 1.01	
Kidneys	17.09 ± 0.91	16.96 ± 1.24	
Transplant R. Vent.	0.15 ± 0.05	0.41 ± 0.07	*
Transplant L. Vent.	0.61 ± 0.08	0.82 ± 0.14	
Total Transplant	0.75 ± 0.12	1.23 ± 0.21	
Brain	2.20 ± 0.11	1.59 ± 0.12	*
Gastrocnemius	0.27 ± 0.08	0.33 ± 0.07	
Skin	7.90 ± 0.58	8.09 ± 1.30	
Carcass	48.27 ± 1.52	51.16 ± 2.63	

"*" means ($p<.05$) in t-test. Expressed as % of C.O. ± standard error, in unanesthetized animals. Lung refers to bronchial fraction and A-V shunts. Liver Art. refers to hepatic artery fraction and portal shunts. Portal refers to combined Spleen, Stomach and Intestine fraction. Carcass is remaining tissue, mostly bone, muscle and fat.

Blood flow per gram of wet organ weight to the liver increased with age, while the flow to the lungs and brain decreased. (see Table 9).

Table 9
Organ Blood Flows
As ml/min/g in Two Age Groups

Organ	5 months n=7	10 months n=6	
Right Ventricle	3.70 ± 0.64	4.08 ± 0.76	
Left Ventricle	4.59 ± 0.60	5.05 ± 0.67	
Total Heart	4.44 ± 0.60	4.90 ± 0.67	
Lungs	2.13 ± 0.45	0.83 ± 0.18	*
Liver Art.	0.06 ± 0.01	0.11 ± 0.02	*
Spleen	3.85 ± 0.46	3.02 ± 0.30	
Stomach	0.75 ± 0.03	0.77 ± 0.09	
Intestine	1.18 ± 0.10	1.22 ± 0.17	
Portal	1.09 ± 0.08	1.10 ± 0.14	
Kidneys	4.86 ± 0.35	4.60 ± 0.15	
Transplant R. Vent.	1.33 ± 0.20	2.10 ± 0.34	
Transplant L. Vent.	1.56 ± 0.15	1.77 ± 0.31	
Total Transplant	1.53 ± 0.15	1.86 ± 0.32	
Brain	0.89 ± 0.05	0.73 ± 0.03	*
Gastrocnemius	0.11 ± 0.03	0.13 ± 0.04	
Skin	0.08 ± 0.01	0.06 ± 0.01	
Carcass	0.16 ± 0.01	0.16 ± 0.02	

"*" means ($p<.05$) in t-test. Expressed as ml/min/g + standard error. in unanesthetized animals. Lung refers to bronchial flow and A-V shunts. Liver Art. refers to hepatic artery flow and portal shunts. Portal refers to combined Spleen, Stomach and Intestine flow. Carcass is remaining tissue, mostly bone, muscle and fat.

3.2.2

Effects of Pentobarbital at Different Ages

Pentobarbital 40-45 mg/kg IP reduced stroke volume, cardiac output and cardiac output per kilogram in the ten months old rat group, compared to control values in unanesthetized animals ($p<0.05$ t-test see Table 10 and Fig 7). No changes due to anesthesia in the general cardiovascular parameters measured could be detected in the five months old rat group. Cardiac output per kilogram in anesthetized rats decreased from 221 ± 65 in the five months old rats to 138 ± 10 ml/min·kg in the ten month old rats ($p<0.05$ t-test).

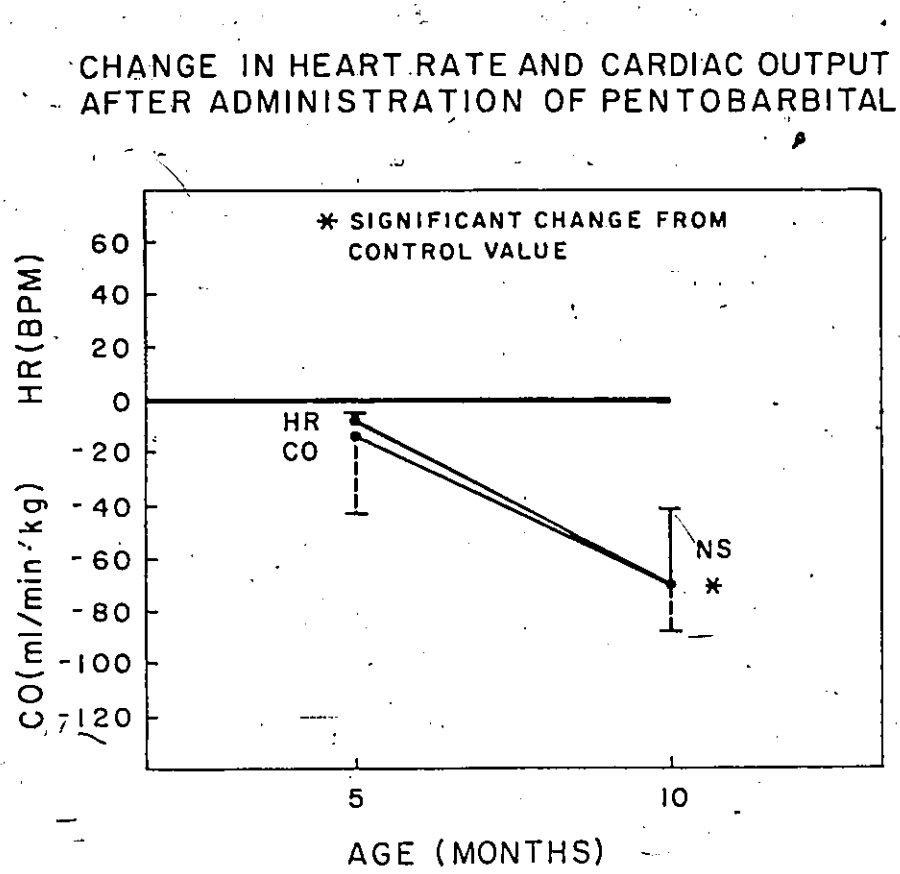


Figure 7. Heart rate appeared to fall and cardiac output fell significantly in only the older age group after administration of 40-45 mg/kg pentobarbital. Presented as mean ± S.E. n = 7 in the younger age group, n = 6 in the older group.

Table 10
General Cardiovascular Parameters
Effect of Age and Pentobarbital Anesthesia

Age group:	5 months n=7	10 months n=6
Heart Rate (BPM)		
Cont.	334 ± 9	369 ± 27
Anes.	324 ± 11	299 ± 14
Diff.	-10 ± 5	-70 ± 29
Stroke Volume (ml)		
Cont.	0.24 ± 0.02	0.27 ± 0.02
Anes.	0.24 ± 0.03	0.22 ± 0.01 *
Diff.	0.00 ± 0.03	-0.05 ± 0.02
Cardiac Output (ml/min)		
Cont.	81 ± 5	99 ± 8
Anes.	77 ± 10	66 ± 1 *
Diff.	-4 ± 11	-33 ± 8
Cardiac Output/kg (ml/min·kg)		
Cont.	232 ± 12	207 ± 16
Anes.	221 ± 25	138 ± 4 * #
Diff.	-12 ± 31	-69 ± 17

"*" means ($p<0.05$) by paired t-test compaired with controls (Cont.) after 45 mg/kg sodium pentobarbital anesthesia (Anes.).
"#" means ($p<0.05$) in unpaired t-test between age groups.
Diff. means difference in means. All values are mean ± standard error in unanesthetized Lewis f/Mai rats. No statistically significant differences (by t-test) were found in the controls.

The fraction of cardiac output to the heart, lungs, liver, intestines and kidneys increased during anesthesia in the five months old animals (see Table 11), relative to control values. The fractions to the spleen, skin and carcass fell in this group. In the ten month old animals the liver and kidney fractions increased, while the spleen, gastrocnemius muscle and carcass fractions fell. The fraction of cardiac output to the lung and intestine in anesthetized rats fell from the five to the ten months old group. The fraction to the transplanted right ventricle and the total heart fraction increased with age ($p<0.01$ and $p<0.05$, respectively, in t-test). The actual values of the cardiac output fractions can be found in Table 17 of the Appendix.

Table 11
Change in Distribution of
Cardiac Output
In Two Age Groups
After Anesthesia
as a Percent of Control

Organ	5 months n=7	10 months n=6
Right Ventricle	(+)115 ± 38 *	(+) 49 ± 18
Left Ventricle	(+) 39 ± 18 *	(+) 13 ± 17
Total Heart	(+) 50 ± 20 *	(+) 17 ± 17
Lungs	(+) 85 ± 26 *	(+)102 ± 38
Liver Art.	(+)1039 ± 173**	(+)767 ± 152**
Spleen	(-) 31 ± 15 *	(-) 39 ± 10 *
Stomach	(+) 2 ± 33	(-) 24 ± 12
Intestine	(+) 38 ± 7 **	(+) 15 ± 8
Portal	(+) 33 ± 10 *	(+) 9 ± 8
Kidneys	(+) 42 ± 8 **	(+) 72 ± 13 **
Transplant R. Vent.	(+) 35 ± 33	(+) 18 ± 19
Transplant L. Vent.	(+) 28 ± 22	(+) 59 ± 45
Total Transplant	(+) 28 ± 20	(+) 45 ± 36
Brain	(-) 8 ± 8	(+) 20 ± 9 #
Gastrocnemius	(-) 54 ± 13	(-) 52 ± 13 *
Skin	(-) 21 ± 7 *	(-) 10 ± 12
Carcass	(-) 43 ± 4 **	(-) 45 ± 2 **

Change in fraction of cardiac output to each organ after pentobarbital ± standard error.
(+) indicates increase from control value.
(-) indicates decrease from control value.
"*" means ($p<0.05$), "**" means ($p<0.01$) significance of difference from control value.
"#" means ($p<0.05$) in t-test between age groups. The increased liver fraction represents a ten fold increase over the control, from about 1% to 10% of the cardiac output.

Blood flow per gram of wet organ weight was also affected by anesthesia in some organs (see Table 12 and Fig 8). The actual values can be found in Table 18 of the Appendix. Hepatic artery flow increased in the young group, while spleen, gastrocnemius muscle and carcass blood flows

fell ($p<0.05$ t-test). In the ten months old group hepatic and renal blood flows increased with anesthesia. Blood flow through the spleen, stomach, intestine, brain and skin fell after pentobarbital ($p<0.05$ t-test). In anesthetized rats myocardial, lung, spleen and carcass blood flows are lower in the ten months old rats then in the five months old group.

Table 12
Change In Organ Blood Flows
In Two Age Groups
After Anesthesia
as a Percent of Controls

Organ	5 months n=7	10 months n=6
Right Ventricle	(+)103 ± 40	(+) 4 ± 18
Left Ventricle	(+) 31 ± 18	(-) 19 ± 19
Total Heart	(+) 41 ± 20	(-) 16 ± 19
Lungs	(+) 86 ± 40	(+) 47 ± 39
Liver Art.	(+)900 ± 83 **	(+)408 ± 85 ** #
Spleen	(-) 38 ± 18 *	(-) 60 ± 5 **
Stomach	(+) 1 ± 36	(-) 49 ± 7 **
Intestine	(+) 35 ± 19	(-) 11 ± 10 *
Portal	(+) 29 ± 20	(-) 26 ± 5 * #
Kidneys	(+) 41 ± 23	(+) 15 ± 2 **
Transplant R. Vent.	(+) 22 ± 38	(-) 17 ± 20
Transplant L. Vent.	(+) 15 ± 13	(+) 17 ± 46
Total Transplant	(+) 15 ± 11	(+) 7 ± 36
Brain	(-) 14 ± 9	(-) 19 ± 6 *
Gastrocnemius	(-) 55 ± 12 *	(-) 63 ± 10
Skin	(-) 19 ± 18	(-) 43 ± 6 **
Carcass	(-) 45 ± 10 **	(-) 62 ± 4 **

Changes in organ blood flows due to pentobarbital ± standard error.
(+) indicates increase from control value.
(-) indicates decrease from control value.
"*" means ($p<0.05$), "**" means ($p<0.01$) significance of difference from control value.
"#" means ($p<0.05$) in t-test between age groups in this table.

EFFECT OF AGE ON ORGAN BLOOD FLOWS
AFTER ADMINISTRATION OF PENTOBARBITAL

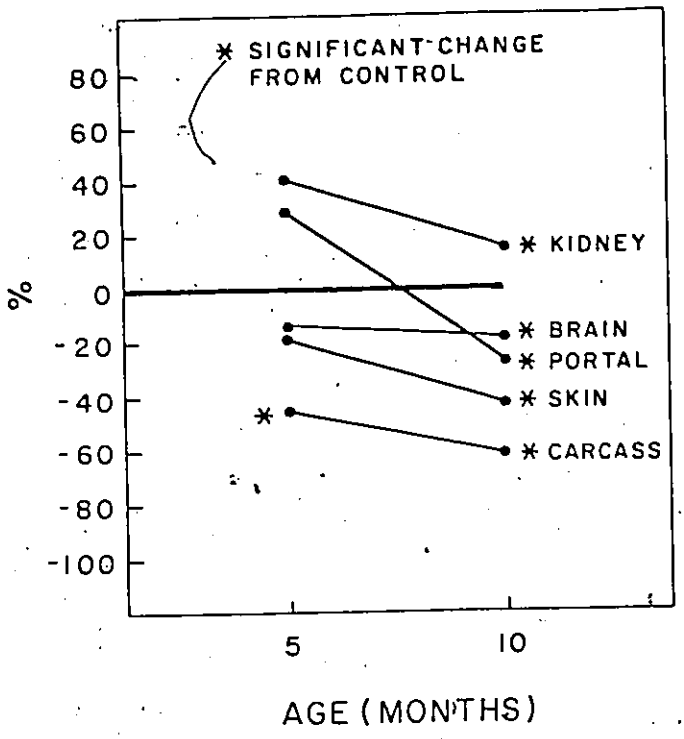


Figure 8. Note that only the renal flow increased after administration of 40-45 mg/kg pentobarbital, significantly only in the older age group. Most other flows fell significantly from unanesthetized controls in only the older age group. The carcass blood flow decreased in both groups. Presented as mean. n = 7 in the younger group, n = 6 in the older age group.

Chapter 4

Discussion

4.1

Effect of Age on Body and Organ Weights

As the fraction of cardiac output to an organ will change with relative changes in the weight of the organ, body weight and organ weights were also measured.

4.1.1

Body Weight

The mean body weight of the three age groups of SD rats were similar. Body weights were significantly different, however, in the five and ten month old Lewis rats of the cardiac transplant study, increasing with age. The former rats all appeared to be obese. This difference stems from the different strains of rat, and possibly the different conditions under which they were raised.

4.1.2

Heart Weight

The heart weight increases during early life until it reaches its adult size. Does it change during senescence? Comparing heart weights alone can be misleading because different body weights are not taken into account, larger individuals having larger hearts. Heart weight must therefore be normalized to another biological variable

before comparing different age groups. Using a heart weight to body weight ratio assumes either a constant body weight, or predictable heart weight - body weight changes. As this may not always be the case, a decrease in this ratio may not always indicate a decrease in heart weight with age.

An ideal reference parameter would reflect relative body size and would not be affected by physiologic, metabolic or environmental factors. Berg and Harmison (1957) showed that tibial length fits this criteria.

Yin and co-workers (1982) found both body weight (prior to senescent weight loss) and tibial length could be used to index body size and to normalize heart weight. They found a 17 % senescent left ventricular hypertrophy when heart weight was normalized with tibial length in 24-26 months old rats compared to 6 months old rats. When heart weight was normalized with body weight instead, an apparent 38 % hypertrophy was noted. They concluded, based on myocyte size measurements, that tibial length was a much better reference of body size with which to quantify organ weight changes. Unfortunately tibial length measurements were not gathered on all rats and this index cannot be used in the present study.

As an alternate to normalizing the heart weights, an empirical formula previously developed in this lab was used to calculate "expected" heart weights derived from the measured body weight.

$$\log (HW) = 0.772 \log (BW) + 0.947$$

where HW is the weight of the two ventricles in mg and BW is the body weight in grams ($r = 0.986$, see = 0.046).

The heart weights found in the five, twelve and twenty four months old SD rats were 1.62 ± 0.05, 1.72 ± 0.21 and 1.70 ± 0.08 grams respectively. They are 127 ± 3 %, 120 ± 10 % and 118 ± 3 % of the expected heart weights.

The weights of the hearts in situ in the five and ten months old Lewis rats are 0.79 ± 0.03 grams and 0.88 ± 0.04 grams, or 98 ± 2 % and 87 ± 3 % of the expected weights. The differences in heart weights and in expected heart weights between the SD and Lewis rats is apparently due to the strain. The SD rats were almost twice as heavy as the Lewis rats and cardiac weight was expected to reflect this.

The actual heart weights within each strain of rat remained relatively unchanged with age in this study. Heart weight appeared to decrease slightly when compared to expected heart weight calculated from the measured body weight. This decrease was not statistically significant however.

Heart weight was found to increase with age by Dyundikova et al (1981) in four age groups of Wistar rats from five to twenty nine months of age. The heart weights relative to body weight did not change appreciably. The reason for this apparent difference with my study may be due to the large number of rats used in the former (58 vs 21). Using larger numbers of rats increases the sensitivity of

statistical tests to detect age differences. ANalysis Of VAriance is a rigorous simultaneous test of differences between groups and should have been used by Dyundikova, rather than the "t" tests she did use. Finally, her oldest animals were about five months older than the ones used by me and this no doubt would affect conclusions drawn regarding the effects of age.

Age related increases in heart mass in Man, both unmodified values and those normalized to surface area, have been reviewed by Lakatta (1979) and Rakusan (1980).

4.1.3

Age and the Weight of the Transplanted Heart

The weights of the transplanted hearts from the 5 months and 10 months old animals are 0.39 ± 0.04 and 0.66 ± 0.03 grams, or 50 ± 6 % and 76 ± 4 % of the recipient heart weights. This apparent age associated increase in heart weight is mainly due to an increase in the transplant right ventricle weight from 0.07 ± 0.02 grams to 0.20 ± 0.02 grams, although the left ventricular weight increased as well ($p<0.001$ t-test for both). This is an atypical result of prolonged heterotopic transplantation (Korecky and Rakusan 1982). The typical transplant right ventricle does not gain weight and the left ventricle loses weight until only 30 % of the original heart mass is left. Inspection of the data reveals that the increased right ventricular mass was consistent in all members of the older group. Possible

reasons for this increase include pulmonary valve disease, mechanical interference of the valve by calcium deposits which are sometimes present, or mechanical trauma of the pulmonary valve during the transplant procedure in the older animals. This would cause resistance to flow or valvular incompetence, resulting in right ventricular hypertrophy.

4.1.4

Weights of Other Organs

The gastrocnemius weight decreased ($p<0.01$ t-test) and stomach weight increased ($p<0.05$ t-test) with age in the two older groups of SD rats. No other changes in organ weights were found in the two older SD rats. All organs increased in weight from the 5 to the 10 months old Lewis rats except the recipient right ventricle, lung, spleen and intestines. These organs did not show significant weight changes.

4.2

Effect of Age on Basic Cardiovascular Parameters

4.2.1

Age and Heart Rate

I found no apparent age related change in basal heart rate both in Sprague Dawley rats from five, twelve and twenty four months and in Lewis f/Mai rats of five and ten months. This study was primarily designed to describe the

old animal. It was not designed specifically to uncover age related trends. A more powerful experimental design for uncovering such trends is to sample at many ages instead of multiple sampling from only three age groups. A regression analysis performed on the data might then uncover any significant trends.

Many authors have found a decrease in resting heart rate with age, in several species (for example: Everett (1958) in pentobarbital anesthetized rats of 300, 540 and 715 days; Vizek and Albrecht (1973) in anesthetized rats of 30-400 days; Woods et al (1978) in neonate sheep to 5 weeks; Roberts and Goldberg (1979) in 1-28 months old conscious rats; Chiueh et al (1980) in conscious rats of 3, 12 and 28 months; Dyundikova et al (1981) in conscious rats from 5 to 29 months; Ohata et al (1981) in conscious rats from 3 to 34 months).

On the other hand Rothbaum and associates (1973) found just the opposite, an increase in heart rate with age. Later they found no age change in heart rate (Rothbaum et al 1974). Abrass and co-workers (1982) also found a slight increase in resting heart rate, which disappeared after the mild stress of a SC injection of saline.

Sato and co-workers (1981) also found no change in the resting heart rate, in supine human males of 15-75 years of age. They found a higher resting rate in upright young males than in older upright males. The difference in heart rates, and the actual upright heart rate, decreased with

age. They suggest that resting autonomic control of heart rate is depressed in older subjects.

Similarly, Strandell (1964) found no age related difference in heart rate, this time in standing 40-80 years old men. The relationship between heart rate and work load was not affected by age but the final heart rate during exercise and the heart rate-workload relationship decreased with age. Likewise, no difference in resting heart rate was found in males and females from 25 to 60 years of age by Montoye and associates (1968), or in the heart rate during excercise. There was, however, an age associated delay in the return to pre-exercise heart rate.

Heart rates decreasing with age were measured in unanesthetized rats by Roberts and Goldberg (1979). They used subcutaneous electrodes and the heart rates measured were considerably higher than those found in the present study using chronic cannulae; ranging from 385 to 440 BPM versus a range of 348 to 374 BPM in the present study. This indicates that the former rats may have been under more stress, and the heart rates may have been recorded under some degree of sympathetic activation rather than under basal conditions. The decrease of heart rate with increasing age (396 to 369 BPM), measured by Chiueh and co-workers (1980), using chronic cannulae were also slightly higher than the measured heart rates in the present study.

Ohata and others (1981) found heart rate to decline in conscious, chronically cannulated Fischer-344

rats from 449 BPM at three months to 332 BPM at thirty four months. These rats, six hours earlier had been anesthetized and their hind quarters had been encased in a plaster cast. These rats must have been under considerable stress.

Dyundikova and associates (1981) used subcutaneous electrodes in conscious rats of five to twenty nine months and found heart rates of 372 BPM in the youngest group, decreasing to 315 BPM in the oldest. As discussed below, some responses to beta receptor stimulation decline with age. This may account for some of the apparent age related declines in heart rate, as placement of subcutaneous electrodes cannot be considered conducive to determination of basal conditions. One can speculate that chronically instrumented, totaly unstressed rats would not show such a decline.

Rothbaum and co-workers (1973) found significantly higher resting heart rates in twenty four months old rats than in twelve months old animals; 331 and 314 BPM, respectively. They measured heart rates by the use of chronic cannulae as in the present study, but after one hour of acclimatization to the measuring apparatus and the laboratory environment. Since the values are slightly lower than in the present study, perhaps our animals were in fact, moderately stressed. Other explanations include strain differences and climate effects.

Experimental conditions often adversely affect results obtained. Vizek and Albrecht (1973) found steadily

decreasing heart rates in anesthetized rats of thirty to four hundred days. As discussed below the effects of anesthesia on the myocardium change with age and probably influenced these results.

There was no difference in heart rates of the three weeks and seven months duration transplants in the 5 and 10 months old rats studied.

4.2.2

Age and Cardiac Output

No age related change in cardiac output was found in the conscious rats in this study. Some other authors, however, have found decreases in cardiac output in both man and the rat (Katori 1979; Luisada et al 1980 in 20-59 years old men; Rothbaum et al 1973 and Rothbaum et al 1974 in conscious 12 and 24 months old rats). A decline in cardiac output with age found in rats by Moustafa and Hopewell (1981) was partially explained by the fact that adult rats increase their body weight by accumulating poorly vascularized adipose tissue. This in effect reduces the circulating blood volume per unit of body weight. My rats on the other hand did not gain weight with age. If cardiac output per unit body weight does decline because of accumulation of adipose tssue, it is not surprising that I found no decline. In the present study an age related fall in cardiac output in anesthetized animals was found and will be discussed in the section dealing with age related effects

of anesthesia. Values for cardiac output found in the present study are close to reported results for rats of unspecified age using various techniques (Rothbaum et al 1973, 86-Rb indicator; Tadepalli et al 1974, direct Fick). Vizek and Albrecht (1973) and McDevitt and Nies (1976) both compare reported cardiac output values with their own obtained by the dye dilution and the microsphere reference sample method, respectively, and found good correlation. The method used therefore, probably does not influence the conclusions drawn about cardiac output changes with age. The present study found none.

4.2.3

Cardiac Output Distribution and Organ Blood Flows

Age associated changes in the distribution of cardiac output in the rat were found. Interpretation of the significance of these findings, both statistical and biological, is now given.

The resolution of the microsphere method as used here is about 0.4 % of the cardiac output if the Buckberg et al (1971) criterion of 400 microspheres per organ is used and 0.2 % of the cardiac output if the Ishise et al (1980) criterion of 200 microspheres is used. The adrenals, gastrocnemius, testes, diaphragm and transplanted right ventricle fail to meet, or only just surpass one or both of the above values. Any blood flow changes occuring in these tissues, even relatively substantial changes, may remain

undetected as the total number of microspheres does not allow comparison of flows to organs with less than 0.2 % (or 0.4 %) of the total cardiac output. The general pattern of the distribution of cardiac output matches that found by other investigators.

4.2.3.1

The Coronary Blood Flow

I found that the coronary fraction of cardiac output and the coronary blood flow normalized for weight, under basal conditions does not appear to vary with age. Normalized coronary blood flow was comparable to that found in other studies on rats and even in studies on other rodents (Peeters et al 1980 in guinea pigs).

Idval and co-workers (1979) while validating their microsphere method, removed the endocardium of each heart in an attempt to avoid including any microspheres trapped in the trabeculae, when measuring coronary blood flow. They found a high correlation (r=0.95) between cardiac output and coronary blood flow, both normalized for weight (Other things being equal, the heart has to work harder to increase cardiac output, and therefore needs a proportionately greater blood flow). They took this high correlation to indicate adequate mixing of the microspheres in the arterial blood. In the present study, the control ventricular blood flows in ml/min per gram of ventricle and cardiac output in ml/min per kilogram of body weight were also found to be

significantly correlated ($p<0.01$, $r=0.66$) without removal of the endocardium. The control ventricular blood flows normalized for weight in both strains of five months old rats were almost identical to those found by Idval and co-workers. The standard deviations were also extremely similar. This means that microspheres are probably not trapped to a significant degree in the trabeculae, and that special preparation of the heart is not necessary.

4.2.3.2

Age and the Transplant Blood Flow

The ventricles of the transplanted rat heart in the 5 months and 10 months old rats received respectively, 0.75 ± 0.33 % and 1.23 ± 0.55 % of the cardiac output, not a statistically significant difference. Blood flow to the ventricles, normalized for weight, was also not significantly different, 1.53 ± 0.39 ml/min·g and 1.86 ± 0.84 ml/min·g, respectively. Pentobarbital anaesthesia, which reduced cardiac output in the 10 months old group by 1/3 did not significantly alter the above values.

When considered by itself, the right ventricle of the transplant did show a difference between the two age groups. It drew a larger fraction of the cardiac output in the 10 months old rats than in the 5 months old ones. It drew this larger fraction both in the anesthetized and unanesthetized groups. Blood flow per unit of mass remained unchanged with either age or anesthesia. Thus, the fraction

of the right ventricle reflects only an increased mass and not a change in perfusion per unit of mass of the ventricle.

As noted earlier, atrophy is present in the left ventricle of the transplant in both age groups, an estimated 35-50 % decrease compared to the recipient left ventricle weights. Atrophy is also present in the transplant right ventricles of the younger age group, an estimated 50 % decrease. As the heart is not doing appreciable external work, at least a 40-50 % decrease in its fraction of cardiac output or a decrease in its blood flow per unit of mass was expected. In fact, the fraction of cardiac output going to the 5 and 10 months old transplanted hearts was 17 % and 29 % of the corresponding recipient fractions, corresponding to a very large decrease. The blood flows per gram were 34 % and 38 % of the recipient blood flows. In 1976 Korecky and co-workers reported that normalized transplant left ventricular blood flow was reduced to only 65 % of the flow to the recipient heart. The 86-Rb uptake method was used in this measurement. Korecky and Rakusan (1982) indicated that this relatively high blood flow value was probably an overestimation due to stagnant blood in the ventricles of the transplanted heart.

The right ventricles of the 10 months old transplant was found to be hypertrophied, possibly due to an increased work load caused by its distention. This is not a typical result of the transplant procedure. It is interesting to note that the blood flow per gram of the

transplant right ventricle is still only about 1/2 of the blood flow per gram of the recipient right ventricle.

Since the blood flow per gram did not change while the weight of the ventricle increased over the 7 month duration of the transplant in the 10 months old rat group, the right ventricle was called upon to perform increased work and did so by hypertrophying rather then increasing contractility. The latter would have increased the blood flow per unit of mass. These conclusions must remain tentative as the minimum number of microspheres criterion was not satisfied in the transplanted right ventricle.

4.2.3.3

The Renal Blood Flow

The renal fraction of cardiac output was found to decrease with age in conscious rats of five, twelve and twenty four months old. The renal blood flow per gram of tissue fell as well.

These results agree with observations in younger anesthetized rats by Yates and Hiley (1979). Using microspheres, they found that 11-12 months old rats had lower kidney flows than 3-4 months old rats and that the fraction of cardiac output decreased as well.

In Man the glomerular filtration rate is very low at birth and reaches mature levels by nine months of age, according to Papper (1978). Tubular function also reaches mature levels by one year. There is then, a progressive

decline in renal plasma flow and GFR after forty years until the flow rate at seventy years of age is about 50 % of the young adult flow. At seventy years of age blood urea nitrogen levels are almost double what they were at forty years, indicating a degree of renal dysfunction. Tubular functions such as concentrating ability, are reduced with age.

Davies and Shock (1950) also found a 53 % decline in effective renal plasma flow between the ages of 20 and 90 years in Man. Similarly, Hollenberg and associates (1974) found a significant age dependent reduction in renal blood flow in normal men, including a larger reduction of flow than of renal mass. They also found that the vasodilatory ability of the kidney, as tested with acetylcholine and with sodium loading, is reduced with age. Together with histological evidence they cite, they conclude that a primary vascular process is involved in the age related fall of renal blood flow.

This fall in renal blood flow with advancing age is opposite to the trend occuring early in the life span. Aperia and Herin (1975) studied renal function in rats of 17-60 days old. From 17 to 30 days, renal blood flow rose slowly. Between 30-60, days it showed a fast rise with no plateau. The kidney fraction of cardiac output increased linearly from the 17 to 60 days covered by this study. Rakusan and Marcinek (1973) using 86-RbCl in unanesthetized growing rats of 1 and 30 days, documented a rise in the

fraction of cardiac output to the kidneys. This was later confirmed by Stulcova (1977), using microspheres and rats of from 9 to 25 days, anesthetized with ether.

The above evidence indicates an increase in renal perfusion with age until adult levels are reached; within a few weeks of birth in rats, and within a year in man. The data presented in this thesis indicate that the fraction of cardiac output to the rat kidney and renal blood flow per gram declines after maturity by up to 50 % in 24 months old rats, compared to rats of 5 months of age. This trend is supported by the clinical studies cited in Man.

4.2.3.4

Age and Blood Flows in Other Organs

Ohata et al (1981) using a [14-C]-iodoantipyrine tracer found that the rat brain blood flow normalized for weight increased between one and three months by about 32 %. From three to twelve months of age the brain blood flow rose by 3-9 % in the various regions measured. From twelve months to twenty four months brain blood flow declined by 17 %. No further change was detected in animals up to thirty four months old.

This type of rise and then fall with age was detected in several organs in the present study, including the fraction of cardiac output to the brain, liver and stomach and the blood flow per gram of small intestine and the portal tissues.

Similarly Dyundikova and co-workers (1981), while looking for an index of ageing in rats, found that several biological parameters also rose and fell with age, reaching a maximum value in the "pre-senile" age group (17-23 months old), rather than in the oldest group of rats (23-29 months of age). These parameters included body weight and heart collagen content.

The significance of this apparent change with age is unclear. It is possibly a result of the type of study, cross sectional rather then longitudinal. Selection in the older groups is undoubtedly occurring, possibly on the basis of the blood flow to some organs. The older age group then becomes a selected subset of the population, different from the younger groups. Mean values for parameters exhibiting this behavior, when cross sectional measurements are made at different ages, may show an increasing trend followed by a decline as selection occurs.

A second possible explanation for the rise and then fall with age of several parameters, is that growth rather then ageing may be involved. The age of five months used in this study to represent the young adult stage was an arbitrary one. It is possible that one or several organ systems were still gaining weight at this time.

The above results indicate the importance of selection of the proper age groups. Previous workers as a whole have used only a "young" age group and an "old" age group. This study on the other hand, using an intermediate

age group, has uncovered trends that would have remained undetected. The advantages of having several groups outweighs the disadvantages, cost and effort.

4.3

The Effect of Age on Some Actions of Isoproterenol

4.3.1

Age, Heart Rate and Cardiac Output

The heart rates in the present study after three minutes infusion of isoproterenol were found to decrease with age ($p<0.05$, linear regression), and the ability of isoproterenol to increase heart rates over control values also decreased with age ($p<0.05$, linear regression). This confirms reports by others of decreased beta responsiveness. Abrass and co-workers (1982) reported a similarly reduced heart rate response in unanesthetized rats of 3, 12 and 24 months of age after 50 µg/kg SC isoproterenol. Yin and others (1979) found a similar decrease in heart rate response to isoproterenol in dogs.

van Brummelen and co-workers (1981) found a greater increase in heart rate after isoproterenol infusion in human subjects less then 25 years of age than in subjects over 50 years old. No difference in this parameter was noticed under control conditions. This corresponds exactly to what I found with rats in this study.

As discussed in the introduction some responses to adrenergic stimulation have been shown to decrease with age (for example Tuttle 1966 decrease in norepinephrine sensitivity in aortic strips from rats of 100-175 days,

340-375 days to 700-800 days; Yin et al 1976 chronotropic response to isoproterenol in man and dog; Kuramoto et al 1979 heart rate in man; Abrass et al 1982 heart rate in rats). Chiueh and co-workers (1980) found that old rats lose their ability to cope with stress, in their case tight restraint, partly due to decreased adrenergic responsiveness.

Kapitola and associates (1973), after administering a very large dose (1 mg/kg IP) of isoproterenol in rats of 190-250 grams, measured cardiac output with the dye dilution method and fractional distribution of cardiac output with Sapirstein's technique. Myocardial uptake of 86-Rb (fraction of cardiac output) after its injection, reached a maximum at 30 minutes and then fell to below control values by 4 hours. Thirty minutes after administration of isoproterenol into anesthetized rats, blood pressure and total peripheral resistance was found to be reduced. Cardiac output and stroke volume per unit of mass had increased. In conscious rats, a large dose of isoproterenol caused blood flow to increase through the heart and lungs and to decrease through the kidneys. The skeletal muscle blood flow non-significantly increased and the spleen, intestine and skin non-significantly decreased. Liver blood flow did not change. These measurements must remain suspect as the fractional distribution of cardiac output was measured in unanesthetized rats, but in order to calculate organ blood

flows, was multiplied by cardiac output values from anesthetized rats.

For the experiments presented in this thesis, isoproterenol was administered IV in a continuous infusion. This is assumed to reach receptor sites more rapidly than an IP or subcutaneous injection. The peak tissue concentration, because of the smaller dose used, was expected to be lower than in most IP or subcutaneous route experiments, but the beta adrenergic level of stimulation should have been at a more physiologic level. This is borne out by the chronotropic effect found in all three age groups. Kapitola and others (1973) used much more massive doses and did not observe an increase in heart rate.

Schroeder et al (1970) found with IV infusions of isoproterenol at 0.05, 0.1, 0.25 and 0.5 µg/min·kg for one to two minutes, that heart rate and cardiac output increased but stroke volume does not. This is confirmed in rats in the present study for isoproterenol infusion at 0.2 µg/min·kg.

In my study on age and isoproterenol, cardiac output, cardiac output per kilogram or per square meter of surface area, but not stroke volume, was shown to increase after isoproterenol in the five months old rats. This was not seen in the two older groups, indicating an age effect on cardiovascular responses to isoproterenol. Note that the dose used probably does produce maximal beta adrenergic stimulation. The increased heart rate measured after

isoproterenol infusion declined with age and the magnitude of the increase over control values declined as well. Cardiac output significantly increased in the five months old rat group after isoproterenol, but did not reach the level of significance in the two older groups in spite of the increased heart rates.

Kuramoto et al (1979) used isoproterenol infusions of 0.02-0.04 µg/min/kg in young and old men, and found a smaller increase in cardiac index as well as reduced diastolic and mean blood pressure in the older group, and concluded that beta adrenergic responsiveness decreased with age.

4.3.2

The Distribution of Cardiac Output and Organ Blood Flows After Isoproterenol

Kapitola et al (1973) measured the fractional distribution of cardiac output in several organs of isoproterenol treated conscious rats but then multiplied these data by cardiac output values taken from anesthetized rats in order to calculate organ blood flows. My experiments on anesthesia show that cardiac output, the distribution of cardiac output, and organ blood flows are altered by anesthesia, and that there is an age effect on these changes. Kapitola's data must be reconsidered with an awareness of these facts.

Debreczeni and co-workers (1980) using 200 gram

anesthetized rats, and the 86-Rb method found that infusion of 0.3 ug/kg/min isoproterenol for five minutes increased cardiac output, as well as blood flows normalized for weight to the heart and carcass. It decreased the fractions of cardiac output to the brain and skin. The blood flow and fraction to the kidney fell as well. In my youngest rats, the cardiac output and the heart and carcass flows also increased after three minutes infusion of 0.2 ug/kg/min. of isoproterenol.

4.3.2.1

Age and the Renal Blood Flow After Isoproterenol

I found decreased renal blood flow after isoproterenol infusion in only the twelve months old rats. The fraction of cardiac output to the kidney after isoproterenol infusion fell in all groups but differed significantly with controls in only the twelve months old group. Kapitola and co-workers also found a decreased renal blood flow and fraction of cardiac output after isoproterenol infusion in rats.

Hoffbrand and co-workers (1973) found when examining effects of isoproterenol in monkeys that the fraction of cardiac output to the kidney fell. These changes are consistent with my results in the two younger age groups.

Rosenblum and others (1968) found in man and in dogs that renal blood flow remains unchanged or decreases

slightly after infusion of enough isoproterenol to increase cardiac output. The fraction of cardiac output to the kidney decreased. They found no change in renal blood flow normalized for surface area and concluded that the beta receptor response is weak or absent in the human and canine kidney.

It has been found by others, and confirmed here, that renal blood flow declines with age from adulthood to senescence. This may be due to structural changes, a regulatory phenomenon or both. The kidney undergoes a maturational increase in blood flow from a very low value at birth, to well into the post neonate period as shown by Rakusan and Marcinek (1973) and Stulcova (1977). Both the very young and the old rat obviously maintain a level of renal perfusion necessary for life. The increased renal perfusion of the middle aged rat may therefore indicate a reserve capacity for kidney function.

The large isoproterenol induced fall in renal perfusion of the middle age group may reflect the expendability of this reserve in times of "stress", induced here by isoproterenol. It appears that beta receptors may be involved in the control of this reserve but not in the maintenance of minimal kidney perfusion.

Alternately, the large decrease in renal blood flow after isoproterenol in middle age rats may be explained, without invoking a reserve mechanism, as follows:

In the five months old animals the myocardial and

carcass flow increased after isoproterenol. In the absence of any other effect this would cause a decrease in the fraction of cardiac output and flow to other tissues, notably the visceral organs including the kidney. The actual increase in cardiac output ensured unchanged blood flow in the visceral organs in these young animals. The distribution of cardiac output did not alter radically.

In the twelve months old group the measured carcass and total heart flows did not increase significantly. Inspection of the data however, reveals major increases in some animals that can be deduced from the higher means and larger standard deviations. Cardiac output values in this group did not significantly increase. Since the circulatory system is closed, an increase in flow to an organ must reduce the fraction of cardiac output to the remaining organs. As a consequence of the slightly increased carcass and heart blood flows, the fraction of cardiac output to most of the visceral organs including the renal fraction fell. The blood flow to the kidneys and small intestine, a very substantial portion of the visceral blood flow, fell.

None of the measured organ blood flows in the twenty four months old rat group significantly changed after isoproterenol infusion. The spleen and portal fraction of cardiac output were reduced from control values. Clearly, the effects of age on the cardiovascular response to isoproterenol infusion are not limited to the heart rate,

the increase of which becomes less with increasing age, and to cardiac output, which increases only in the young adult group. Tissue blood flows after isoproterenol are affected by the response of the vascular smooth muscle that shows regional differences in the effects of age.

This regional difference in the effects of age may explain why the large kidney fraction and renal blood flow decrease occured in the middle age group and not in the young adult or the senescent rat. The myocardial and carcass blood flows increased in the two youngest groups, but the cardiac output increase needed to maintain blood flows elsewhere, occured only in the youngest group.

4.3.2.2

Age and Muscle Blood Flows After Isoproterenol

Gastrocnemius blood flows tended to increase after isoproterenol infusion in all three age groups but the only statistically significant increase occured in the middle age group.

Carcass blood flow, a large portion of which is skeletal muscle blood flow, increased significantly in the youngest age group, tended to increase, but not significantly, in the middle age group, and did not change in the oldest rats.

Blood flow to the diaphragm, in contrast, remained remarkably unaffected by isoproterenol. This difference may be due to the different functional demands made on each

muscle. In an undisturbed rat, muscles of the carcass do not demand a high blood flow. On the other hand, the diaphragm is working closer to its maximum capacity and therefore draws more blood per gram of tissue. The increased blood flow induced by isoproterenol in the gastrocnemius and carcass, indicates a beta adrenergic dependent ability to increase blood flow that is apparently reduced or absent in the diaphragm.

van Brummelen and co-workers (1981) found smaller forearm blood flow increases due to isoproterenol infusion in older persons than in younger persons. The forearm blood flow is made up largely of muscle blood flow.

Isoproterenol challenge has been tested for use in place of exercise stress testing in the diagnosis of ischemic heart disease in aged humans (Kuramoto et al 1978). Kuramoto and co-workers (1979) compared hemodynamic effects of exercise (50 and 100 W) with isoproterenol infusion (0.02 and 0.04 ug/min/kg for 5 minutes) in normal young and elderly men. They found decreased heart rate, cardiac index and blood pressure response to isoproterenol in the elderly group compared to the young group. The responses to exercise on the other hand, were similar in the two groups. The decreased responses to beta adrenergic stimulation are directly comparable to my results.

Blood flow in rat leg muscles, including the gastrocnemius, was measured by Laughlin and Armstrong (1982). Using a technique similar to the one used by me,

they measured blood flow in rats on a treadmill before exercise and at several running speeds. The gastrocnemius blood flow in these trained rats anticipating exercise was remarkably close to that measured by my self after isoproterenol infusion, 0.32 ml/min·g of middle gastrocnemius versus 0.28-0.31 ml/min·g in my rats. Other determinations on truly resting rats mentioned in their discussion are close to control levels which I measured.

There seems little doubt that the functiopal response to beta adrenergic stimulation is decreased with age to different degrees in different tissues, including the heart, kidney and some skeletal muscle. Age related studies must be designed in such a manner that sympathetic stimulation is avoided, as the response to beta receptor stimulation changes with age, generally decreasing.

4.4

Effect of Age on Some Actions of Pentobarbital

To this author's knowledge, no age related differences in the cardiovascular actions of pentobarbital anesthesia have been reported elsewhere. All citations refer to differences among anesthetized animals of different ages or to differences between anesthetized and unanesthetized animals at a single age, but not to both. As it turns out, differences were found to exist in the cardiovascular response to pentobarbital between two age groups. This finding is expected to stimulate more comprehensive work in this area.

4.4.1

Heart Rate and Cardiac Output After Anesthesia

In this study pentobarbital (45 mg/kg IP) decreased cardiac output, and cardiac output per kilogram in the ten months old animals compared to the unanesthetized controls. General cardiovascular parameters, including heart rate, remained unchanged in the five months old group after pentobarbital anesthesia.

Cardiac output per kilogram in pentobarbital anesthetized animals was found to decrease with age from the five months old to the ten months old rats. The actual cardiac output did not change with age. Yates and Hiley

(1979) found similar results after ketamine anesthesia in their three to four months and eleven to twelve months old rats. Dyundikova and others (1981) also found cardiac output and cardiac output per kilogram to fall with age in ether anesthetized rats of 5 to 29 months of age.

Ericsson (1971) found a slightly reduced cardiac output in adult dogs after administration of pentobarbital. Popovic and Kent (1964) found a decrease in cardiac output per kilogram due to pentobarbital in young adult rats.

The above studies indicate that anesthesia induces a fall in cardiac output over unanesthetized controls. Age has been found in some studies to augment this effect.

4.4.2

The Distribution of Cardiac Output and Organ Blood Flows After Anesthesia

The fraction of cardiac output to the heart, lungs, liver, intestine, portal and renal tissues increased after anesthesia in the five months old animals at the expense of the spleen, skin and carcass fractions. In the ten months old animals the fractions to the liver and kidneys increased and the fractions to the spleen, gastrocnemius and carcass fell.

The pentobarbital increased liver fraction of the cardiac output is the most striking finding. It may be an artifact, as the comparisons were made with control values that appear slightly lower then those control values found

in the SD rats of the isoproterenol study. The values are not very much lower however, and there is no reason to suspect a problem with the methodology. Perhaps instead, the increased liver fraction represents a functional hyperemia as the liver attempts to detoxify the animal of pentobarbital. In the same vein, the increased kidney fractions may be due to the comparable processes.

Similar increases in the fractions of cardiac output going to the lung, pancreas and GI tract in the young adult rat were found by Sasaki and Wagner (1971). They also found pentobarbital to decrease the fraction of cardiac output to the brain while increasing the spleen fraction of cardiac output.

Rakusan and Blahitka (1974), using 25 μm in diameter microspheres, also found pentobarbital anesthetized adult rats exhibited a decrease in the fraction of cardiac output to the heart and brain, compared to unanesthetized controls. They found an increase in the cardiac output fraction to the kidney and skin.

Aardal and associates (1973) determined that pentobarbital in rabbits caused a redistribution of blood from the skeletal muscles to the kidneys and intestines. They used a technique similar to the microsphere method in which 20-50 micrometer in diameter macroaggregates of 131-I labelled albumin were used.

A redistribution of cardiac output in the monkey after sodium pentobarbital was found by Forsyth and

Hoffbrand (1970). The fraction to the lung and kidney increased and the brain fraction fell. The increased lung fraction after anesthesia remained after injection of the microspheres into the abdominal aorta, and was thought to be due to the opening of arterio-venous shunts. Cardiac output fell but was not significantly different from unanesthetized values.

Richardson and Withrington (1981) in their review, noted that in the rat, anesthetics slightly increases the hepatic arterial and total liver blood flow.

These studies indicate a shift of cardiac output to the visceral organs from skeletal muscle and brain occurs in response to anesthesia. What previously was not known is whether age changes this redistribution. The answer now appears to be 'yes'.

In my study on anesthesia, in the five months old animals blood flow per gram was found to increase in the liver and the flow to decrease in the spleen, gastrocnemius and carcass. In the ten months old animals, liver and kidney flows increased and brain, spleen, stomach, intestinal, portal, skin and carcass blood flows fell.

As indicated in the previous section, the increases in the blood flow to the liver and kidney may be due to a functional hyperemia as the rats attempt to clear the pentobarbital from their systems.

Yates and Hiley found their GI tract fractions to increase and the lung fraction to decrease with age in rats

anesthetized with ketamine. They also found decreased organ blood flows with age in the liver, kidney, skin and skeletal muscle in anesthetized rats. Blood flows in the present study decreased with age after anesthesia in the heart, lungs, spleen and carcass. This difference is probably due to the different effects of pentobarbital (present study) and ketamine anesthesia (Yates and Hiley), and possibly due to different circulatory effects of the prone position (present study) and the dorsal recumbent position (Yates and Hiley).

4.4.2.1

Coronary Blood Flow After Anesthesia

Coronary blood flow in the Lewis rats treated with pentobarbital showed a non-significant increase in the 5 months old group. The ten months old group exhibited a non-significant drop. Rubanyi and Kovach (1980) found in isolated perfused hearts taken from unanesthetized and from anesthetized rats, greater blood flows in the anesthetized group, 5.0 $\pm$ 0.9 ml/min versus 8.6 $\pm$ 0.8 ml/min respectively, and decreased vascular resistance. The spontaneous heart rate of their preparation was higher after anesthesia as well. They concluded that the isolated heart is affected profoundly by previous treatment with pentobarbital. Blood flow control mechanisms that are not localized in the heart are therefore involved in the control of the coronary circulation under anesthesia in the intact

animal.

The fraction of cardiac output to the transplanted heart in both five and ten months old rats is increased slightly, but not significantly. This occurs in the face of a decreased cardiac output in the older group. The blood flow per gram of transplanted ventricle does not change.

4.4.2.2

Arterio-venous Shunting

The apparent bronchial blood flow per gram of tissue decreased with age in conscious and anesthetized Lewis rats in the present study. In the light of the Forsyth and Hoffbrand study this could be interpreted as an age related decrease in arterio-venous shunting. This trend is not found in the SD rats of the isoproterenol stress study. It therefore is either a strain difference, or alternately, the result of shunting of blood through the three weeks old transplant. If the latter is the case, shunting seems to stop between 3 weeks and 7 months of transplant duration.

The apparent liver arterial blood flow per gram increased with age in 5 to 12 months old and 5 to 10 months old conscious rats but the greatly increased values did not show this trend in anesthetized rats. This may also be an example of arterio-venous shunting of blood (and microspheres) through the splanchnic organs, this time due to anesthesia, and not age.

4.4.2.3

Brain Blood Flow After Anesthesia

In the rats of the age and anesthesia study, brain blood flow fell with age in both awake and anesthetized animals. This finding is partially confirmed by the finding of Ohata and co-workers (1981) that the cerebral blood flow in most regions decreased with age after twelve months in conscious rats.

4.4.2.4

Renal Blood Flow After Anesthesia

Koppen and co-workers (1979) found a decreased total renal blood flow after pentobarbital or Inactin anaesthesia in the tracheostomized rat compared with unanesthetized controls. Their reference sample method was extremely similar to the one used by myself with the exception that the microsphere suspending medium they used contained 10 % dextran. This is known to cause almost immediate severe hypotension. Their anaesthetic associated drop in renal blood flow therefore may be an artifact of their method.

The present study in contrast, shows an anesthesia associated increase in the renal fraction of cardiac output in both age groups. The blood flow per gram of kidney in the ten months old rats increased significantly over unanesthetized controls. As each rat served as its own

control, my study should more closely reflect actual effects of anesthesia than some previous studies in which an anesthetized group and a different unanesthetized control group were used. On the other hand, the rats may have been overhydrated during the operative procedure as a result of flushing of the cannulae, and then may have become slightly dehydrated during the recovery period, although water was freely available. This would result in an apparent increased renal blood flow due to anesthesia, if in fact there was an increased renal blood flow due only to the decreased plasma osmolarity.

Supporting the first interpretation, Rauch and Beatty (1977) using Sapirstein's method found a pronounced redistribution of cardiac output from the skeletal muscles to the abdominal viscera in bats and mice. The kidney and carcass flows increased while the liver flow did not. Rakusan and Blahitka (1974) also found increased kidney fractions after anesthesia in rats.

Cupples and co-workers (1982) have recently published apparently contradictory results, finding no change in renal blood flow due to anesthetics, including pentobarbital. They report a fall in blood pressure which they attribute to anesthesia, but also may be due to the first microsphere injection. No mention of the microsphere suspending medium was made. If present, dextran could have influenced their results.

4.4.3

Mechanism for Age Related Blood Flow Changes After Anesthesia

Are we looking at a change with age of individual organ responses to anesthesia, at a change with age of central nervous control of circulation, or at some other mechanism?

The decreased heart rate, stroke volume and cardiac output in the ten months old group after pentobarbital anesthesia points to myocardial depression in the older group as a major contributor to the age related differences. The high mortality in twelve and twenty four months old rats after pentobarbital, experienced by me during unreported methodological studies supports this view. An age related increase in the sensitivity of the myocardium to pentobarbital would explain the decreased cardiac output and stroke volume in the older group.

In his book, Prys-Roberts (1980) states that all anaesthetics directly depress the contractile function of myocardial cells. Pentobarbital seems to uncouple sarcoplasmic reticular calcium binding from ATPase activity. My study indicates that this effect may be age dependent.

The decreased cardiac output in turn forces various organ autoregulatory mechanisms into play.

In conclusion, pentobarbital anesthesia caused a decrease in cardiac output, probably due to myocardial

depression in ten months old rats, whereas cardiac output did not change in the five months old rats. Hepatic and renal blood flows in both age groups increased after administration of pentobarbital (40 mg/kg). Blood flow to the heart and spleen in anesthetized animals decreased with age in the two groups studied. An apparent decrease in blood flow to the lung in the older group may indicate a decrease in arteriovenous shunting with age, possibly through the transplanted heart. It should be remembered that the older rats were significantly heavier than the younger rats. If weight gain occured by an increase in adipose tissue then the older group may have recieved a larger "lean weight dose" than the younger age group. Although perhaps less interesting to a physiologist than other types of age related changes, this nevertheless is an important observation.

Chapter 5

Summary and Conclusions

Heart rate was found to remain constant with age in SD rats from five to twenty four months. The apparent decreases found by others may be due to either the effect of anesthesia or to the decreasing effectiveness of the beta adrenergic response to stress.

Cardiac output did not appear to change with age. The distribution of cardiac output, however, is affected by the age of the animal. A gradual decrease in both the renal and splenic fractions of cardiac output is the most notable finding. Other organs show a rise and then a fall in their fractions. The reasons for this behaviour are unclear.

While some organ blood flows normalized for weight are altered with different ages, only the renal blood flow shows a definite trend, decreasing to about half the original value in the oldest group.

Administration of equivalent doses of isoproterenol to rats produced a spectrum of effects in the three age groups. The youngest group generally showed the strongest responses, especially in increased heart rate, cardiac output and cardiac output normalized for body weight. Only the heart rate increased significantly in the oldest group and the magnitude of the increase over control values was less than in the younger groups.

Blood flow to the ventricles of the heart after isoproterenol increased significantly over control values in only the youngest group. In contrast, the renal blood flow after isoproterenol greatly fell in only the middle age group. This may be explained by the following. In the youngest age group, the kidney can compensate for the vasodilation in other tissues that otherwise would deprive it of part of its flow. The kidney in the middle age group apparently can not. No significant changes from control blood flow values were found in any organ in the oldest age group, although the spleen and portal fractions of cardiac output were slightly reduced.

The changing effects of isoproterenol administration at different ages may be explained largely by an asynchronously decreasing functional beta adrenergic response. The questions of whether the number of beta receptors changes, if the structure of the receptor itself changes with age, or if some other mechanism in the steps of isoproterenol action is affected by age, cannot be answered by these experiments.

Equivalent doses of pentobarbital decreased stroke volume, cardiac output and cardiac output per kilogram in ten months old Lewis rats but not in five months old rats. Additional support for age related differences comes from the high mortality experienced in preliminary studies on twelve and twenty four months old SD rats due to pentobarbital anesthesia. Since the beta adrenergic

response to stress declines with age, and cardiovascular effects of anesthesia are partially compensated for by sympathetic system activation, heart rate in anethestized older animals is expected to be reduced.

A similar argument may be advanced for age related changes of the cardiac output after anesthesia. Anesthesia increasingly depresses the myocardium and lowers cardiac output with increasing age. The effectiveness of beta adrenergic stimulation, part of the reflex mechanisms normally used in compensating for this myocardial depression, is reduced with age. Adrenergic stimulation therefore increases cardiac output more in younger age groups then in older age groups.

From evidence gathered in the two age groups examined and by the indirect evidence of the anesthetic deaths in only the two older age groups of the preliminary isoproterenol study, it may be concluded that pentobarbital anesthesia profoundly affects the aged cardiovascular system, probably through the mechanism of myocardial depression. This does not explain the greatly increased renal and hepatic fractions and flows in both age groups. Perhaps this is an example of functional hyperemia due to the actions of both of these organs to detoxify the anesthetic agent.

The transplanted hearts recieved 17 % to 29 % of the fraction of cardiac output the recipient hearts recieved in the two age groups. Blood flow per gram was 34 % and 38

% of the host heart flow. Both sets of the above values are not significantly different between the five and twelve months old age groups. An apparent age related increase in the weight of the transplanted heart was probably the result of right ventricular hypertrophy in the older hearts. This is not the typical atrophy response in transplanted non-working hearts under study in this lab.

A valuable lesson may be derived from this study. Any apparent age related difference in a parameter must be carefully studied before conclusions are drawn. Special attention must be given to measuring parameters under truly basal conditions. As shown here, beta agonist effectiveness declines with age and cardiovascular effects of pentobarbital increase with age. Both of these factors may have influenced the conclusions of age related studies conducted previously.

A second lesson to be taken is the importance of intermediate age groups in research on aging. Several examples of transient changes in organ fractions of cardiac output or blood flows were discovered in this study, including the rise and then fall of brain, liver and stomach fractions and the renal blood flow response to isoproterenol. These would have been missed had only a "young" group and an "old" group been used.

In conclusion, this study has shown that there are indeed age related changes in the distribution of cardiac output in the normal rat, most importantly a decreased renal

fraction. Also shown was the fact that sympathetic system activation may well have influenced some studies in the past through the age related decreased responses to beta adrenergic stimulation. Pentobarbital anesthesia also may have misled investigators as it produced dramatic changes in cardiac output and its distribution in only the older group of two examined. The importance of intermediate age groups in aging research was noted. Finally, blood flow to the isotransplanted non-working rat heart was found to be reduced to about 35 % of the recipient heart blood flow.

References

Aardal, N.P., K. Svanes and K.E. Egenberg
Effect of hypothermia and pentobarbital anaesthesia on the distribution of cardiac output in rabbits. Eur. Surg. Res. 5: 362-372, 1973.

Abrass, I.B, J.L. Davis and P.J. Scarpace
Isoproterenol responsiveness and myocardial beta-adrenergic receptors in young and old rats. J. Gerontol. 37 (2): 156-160, 1982.

Altura, B.M. and B.T. Altura
Some physiological factors in vascular reactivity 1 Ageing in vascular smooth muscle and its influence on vascular reactivity. Carrier Jr. O. and S. Shebata (eds.), Igaku-Shoin, Tokyo, pp 169-188, 1977.

Amery, A., H. Wasir, C. Bulpitt, J. Conway, R. Fagard, P. Lijen and T. Reybrouck Aging and the cardiovascular system. Acta Cardiol. 33 (4): 443-467, 1978.

Aperia, A. and P. Herin
Development of glomerular perfusion rate and nephron filtration rate in rats 17-60 days old. Am. J. Physiol. 228 (5): 1319-1325, 1975.

Archie, J.P., D.E. Fixler, D.J. Ullyot, J.I.E. Hoffman, J. Rutley and E.L. Carlson Measurement of cardiac output with and organ trapping of radioactive microspheres. J. Appl. Physiol. 35: 148-154, 1973

Bartrum, R. Jr., D. Bukowitz and N.K. Hollenberg
A simple radioactive microsphere method for measuring regional flow and cardiac output. Invest. Radiol. 9: 126-132, 1974.

Bender, A.D
The effect of increasing age on the distribution of peripheral blood flow in Man. J. Am. Geriatrics Soc. 13 (3): 192-198, 1965.

The influence of age on the activity of catecholamines and related therapeutic agents. J. Am. Geriatrics Soc. 18(3): 220-232, 1970.

Berg, B.N and C.R. Harmison
Blood pressure and heart size in aging rats. J. Gerontol. 10: 416-419, 1955.

Growth, disease and aging in the rat. J. Gerontol. 12: 370-377, 1957.

Blahitka, J. and K. Rakusan
Blood flow in rats during hemorrhagic shock: differences between surviving and dying animals. Circ. Shock 4: 79-93, 1977.

Brandfonbrener, M., M. Landowne and N.W. Shock
Changes in cardiac output with age. Circulation 12: 557, 1955.

Buckberg, G.D., J.C. Luck, D.B. Payne, J.I.E. Hoffman, J.P. Archie and D.E. Fixler Some sources of error in measuring regional blood flow with radioactive microspheres. J. Appl. Physiol. 31 (4): 598-604, 1971.

Charbon G.A. and R.S. Reneman
The effects of beta-receptor agonists and antagonists on regional blood flow. Eur. J. Pharmacol. 9: 21-26, 1970.

Chiueh, C.C., S.M. Nespor and S.I. Rapoport
Cardiovascular, sympathetic and adrenal cortical responsiveness of aged Fischer-344 rats to stress. Neurobiology of Ageing 1 (2): 157-163, 1980.

Clausen, G., A. Kirkebo, I. Tyssebotn, E.S. Ofjord and K. Aukland Erroneous estimates of intrarenal blood flow distribution in the dog with radiolabeled microspheres. Acta Physiol. Scand. 107: 385-387, 1979.

Cobbold, A.F., J. Gensburg and A. Paton
Circulatory, respiratory and metabolic responses to isopropylnoradrenaline in Man. J. Physiol. 151: 539-550, 1960.

Cooper, T.G.
Surgical sympathectomy and adrenergic function. Pharmacol. Rev. 18: 611-618, 1966.

Cotchin, E and F.J.C. Roe (eds.)
Pathology of Laboratory Rats and Mice, Blackwell Scientific Publications, Oxford, 1967.

Cupples, W.A., A.T. Veress and H. Sonnenberg
Lack of effect of barbiturate and ketamine anesthesia on renal blood flow in chronically instrumented rats prepared for micropuncture. Can. J. Physiol. Pharmacol. 60: 204-207, 1982.

Davies, D.F. and N.W. Shock
Age changes in glomerular filtration rate, effective

renal plasma flow, and tubular excretory capacity in adult males. J. Clin. Invest. 24(5): 496-507, 1950.

Debreczeni, L.A., Cs. Farsang and L. Takas
Effect of phenoxybenzamine, propanolol, phenylephrine and isoproterenol on the circulation of rats bearing Guerin carcinoma. Acta Physiol. Acad. Sci. Hung. 56 (4): 341-365, 1980.

Debreczeni, L.A. and T. Fenyvesi
Effect of adrenoreceptor blocking compounds (propanolol, practolol and LB 46) on isoprenaline induced changes in regional blood flow in the rat. Br. J. Pharmacol. 41: 171-176, 1971.

Dyundikova, V.A., Z.K. Silvon and T.L. Dubina
Biological age and its estimation 1 Studies of some physiological parameters in albino rats and their validity as biological age tests. Exp. Gerentol. 16: 13-24, 1981.

Ericsson, B.
Effect of pentobarbital sodium anasthesia as judged with aid of radioactive carbonized microspheres on cardiac output and its fractional distribution in the dog. Acta Chir. Scand. 137: 613-620, 1971.

Ericsson,E. and L. Lundholm
Adrenergic beta receptor activity and cyclic AMP metabolism in vascular smooth muscle; variations with age. Mech Ageing Dev. 4: 1-16, 1975.

Everitt, A.V.
The electrocardiogram of the ageing male rat. Gerontologia 2: 204-212, 1958.

Flaim, S.F., Z.Q. Morris and T.J. Kennedy
Dextran as a radioactive microsphere suspending agent: severe hypotensive effect in rat. Am. J. Physiol. 235 (Heart Circ. Physiol. 4): H587-H591, 1978.

Fleisch, J.H.
Further studies on the effect of ageing on beta-adrenoceptor activity of rat aorta. Br. J. Pharmacol. 42: 311-313, 1971.

Age-related changes in the sensitivity of blood vessels to drugs. Pharmacol. Ther. 8: 477-487, 1980.

Fleisch, J.H. and C.S. Hooker
The relationship between age and relaxation of vascular smooth muscle in the rabbit and rat. Circ. Res. 38:

243-249, 1976.

Fleisch, J.H., H.M. Maling and B.B. Brodie
Beta-receptor activity in rat aorta. Variations with age and species. Circ. Res. 26: 151-162, 1970

Fleisch, J.H. and S.M. Spaethe
Vasodilation and ageing evaluated in the isolated perfused rat messenteric vascular bed: preliminary observations on the vascular pharmacology of Dobutamine. J. Cardiovasc. Pharmacol. 3: 187-196, 1981.

Forsyth, R.R and B.I. Hoffbrand
Redistribution of cardiac output after sodium pentobarbital anesthesia in the monkey. Am. J. Physiol. 218: 214-217, 1970.

Forsyth, R.P., A.S. Nies, F. Wyler, J. Neutze and K. Melmon Normal distribution of cardiac output in the unanesthetized restrained rhesus monkey. J. Appl. Physiol. 25 (6): 736-71, 1968.

Foster, D.O. and M.L. Frydman
Comparison of microspheres and 86-Rb+ as tracers of the distribution of cardiac output in rats indicates invalidity of 86-Rb+ based measurements. Can. J. Physiol. Pharmacol. 56: 97-109, 1978.

Frolkis, V.V., V.V. Bezrukov, L.N. Bogalskaya, N.S. Verkhratsky, V.P. Zamoslian, V.G. Shevtchuk and I.V. Shlchegoleva Catecholamines in the metabolism and functions regulation in aging. Gerontologia 16: 129-140, 1979.

Gerstenblith, G., E.G. Lakatta and M.L. Weisfeldt
Age changes in myocardial function and exercise response. Prog. Cardiovasc. Diseases 19 (1): 1-21, 1976.

Gey, K.F., W.P. Burkard and A. Pletscher
Variation of the norepinephrine metabolism of the rat heart with age. Gerontologia 11: 1-11, 1965.

Green, J.F.
Mechanism of action of isoproterenol on venous return. Am. J. Physiol. 232: H152-H156, 1977.

Greenberg, L.H. and B. Weiss
Beta-adrenergic receptors in aged rat brain: reduced number and capacity of pineal gland to develop supersensitivity. Science 201: 61-63, 1978.

Ability of aged rats to alter beta adrenergic receptors of brain in response to repeated administration of desmethylimipramine. J. Pharmacol. Exp. Ther. 211: 309-316, 1979.

Greenway, C.V. and V.S. Murthy
Effects of vasopressin and isoprenaline infusion on the distribution of blood flow in the intestine: criteria for the validity of microsphere studies. Br. J. Pharmacol. 46: 177-188, 1972.

Guarnieri, T., C.R. Fliburn, G. Zitnik, G.S. Roth and E.G. Lakatta Contractile and biochemical correlates of beta-adrenergic stimulation of the aged heart. Am. J. Physiol. 239 (Heart Circ. Physiol. 8): H501-H508, 1980.

Gulati, O.D., B.P. Mathew, H.M. Parckh and V.S.R. Krishnamarty Beta adrenergic receptor of rabbit thoratic aorta in relation to age. Jpn. J. Pharmacol. 23: 259-268, 1973.

Hales, J.R.S.
Radioactive microsphere measurement of cardiac output and regional tissue blood flow in the sheep. Pflgers Arch. 344: 119-132, 1973.

Radioactive microsphere techniques for studies of the circulation. Clin. Expt. Pharmacol. Physiol. Supp 1: 31-46, 1974.

Hayashi, S., M. Miyazaki and N. Toda
Age dependent changes in the response of isolated rabbit cerebral arteries to vasoactive agents. Jap. J. Pharmacol. 27 (suppl): 108, 1977.

Heyman, M.A., B.D. Payne, J.I.E. Hoffman and A.M. Rudolph Blood flow measurements with radionuclide labeled particles. Prog. Cardiovasc. Diseases 20 (1): 55-79, 1977.

Hoffbrand, B.I., R.P. Forsyth and K.L. Melmon
Dose-related effects of isoprenaline on the distribution of cardiac output and myocardial blood flow in conscious monkeys. Cardiovasc. Res. 7 (5): 664-669, 1973.

Hollenberg, N.K., D.F. Adams, H.S. Solomon, A. Rashid, H.L. Abrams and J.P. Merriff Senescence and the renal vasculature in normal man. Circ. Res. 34: 309-316, 1974.

Hruza, Z, and B.W. Zweifach
Effect of age on vascular reactivity to catecholamines in rats. J. Gerontol. 22: 469-473, 1967.

Idvall, J., K.F. Aronsen, L. Nilsson and B. Nosslin
Evaluation of the microsphere method for determination of cardiac output and flow distribution in the rat. Eur. Surg. Res. 11 (6): 423-433, 1979.

Imai, Y., K. Saloh and N. Taira
Role of the peripheral vasculature in changes in venous return caused by isoproterenol, norepinephrine and methoramine in anesthetized dogs. Circ. Res. 43: 553-561, 1978.

Imms, F.J., R.L.B. Neame and D.A. Powis
Responses of the cardiovascular system of the rat to alpha- and beta- adrenoceptor agonists. Br. J. Pharmacol. 60: 107-114, 1977.

Ishise, S., B.L. Pegram, J. Yamamoto, Y. Kitamura and E.D. Frohlich Reference sample microsphere method: cardiac output and blood flows in conscious rat. Am. J. Physiol. 239 (Heart Circ. Physiol. 8): H443-H449, 1980.

Kaihara, S., P.D. van Heerden, T. Migita and H.N. Wagner Jr. Measurement of distribution of cardiac output. J. Appl. Physiol. 25 (6): 696-700, 1968.

Kapitola, J., F. Kolbel, M. Schullerova, and O. Schreiberova Total and local circulatory figures in rats. Sb. Lek. 72 (10): 276-280, 1970.

Blood flow in rat organs during their development [in Czech]. Sb. Lek. 73: 143-148, 1971.

Regional blood flow of myocardium and other rat tissues after isoproterenol administration. Physiol. bohemoslov. 22 (2): 137-142, 1973.

Katori, R.
Normal cardiac output in relation to age and body size. Tohoku J. Exp. Med. 128 (4): 377-387, 1979.

Kelliher, G.J. and S.T. Conahan
Changes in vagal activity and response to muscarinic receptor agonists with age. J. Gerontol. 35 (6): 842-849, 1980.

Kety, S.S.

The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol. Rev. 3: 1-41, 1951.

Knoebel, S.B., D.K. Lowe, D.E. Lovelace and J.J. Freidman Myocardial blood flow as measured by fractional uptake of Rubidium-84 and microspheres. J. Nucl. Med. 19 (9): 1020-1026, 1978.

Koeppen, B.M., A.I. Katz and M.D. Lindheimer Effect of general anaesthesia on renal hemodynamics in the rat. Clin. Sci. 57: 469-471, 1979.

Korecky, B., J.J. Blahitka and H.A. Heggtveit Heterotopically isotransplanted empty beating rat heart with preserved coronary circulation. Physiol. Can. 7: 39, 1976.

Korecky, B. and K. Rakusan Morphological and physiological aspects of cardiac atrophy. in BIOLOGY OF CARDIAC HYPERTROPHY AND FAILURE, Raven Press, in press.

Kuramoto, K., S. Matsushita, I. Kuwajima, T. Iwasaki and M. Murakami Comparison of hemodynamic effects of exercise and isoproterenol infusion in normal young and old men. Jpn. Circ. J. 43: 71-76, 1979.

Kuramoto, K., S. Matsushita, J. Mifune, M. Sakai and M. Murakami Electrocardiographic and hemodynamic evaluation of isoproterenol test in elderly ischemic heart disease. Jpn. Circ. J. 42: 955-960, 1978.

Lakatta, E.G. Alterations in the cardiovascular system that occur in advanced age. Fed. Proc. 38: 163-167, 1979.

---------- Age-related alterations in the cardiovascular response to adrenergic mediated stress. Fed. Proc. 39: 3173-3177, 1980.

Lakatta, E.G., G. Gerstenblith, C.S. Angell, N.W. Shock and M.L. Weisfeldt Diminished inotropic response of aged myocardium to catecholamines. Circ. Res. 36: 262-269, 1975.

Langer, S.Z. The role of alpha- and beta- presynaptic receptors in the regulation of noradrenaline release elicited by nervous stimulation. Clin. Sci. Med. 51: 423s-426s, 1976.

Laughlin, M.H. and R.B. Armstrong

Muscular blood flow distribution patterns as a function of running speed in rats. Am. J. Physiol. 243 (Heart Circ. Physiol. 12): H296-H306, 1982.

Lin, Y.C., C.A. Dawson and S.M. Horvath
The expression of cardiac output in the albino rat, Rattus rattus. Comp. Biochem. Physiol. 33: 901-909, 1970.

Luisada, A.A., P.K. Bhat and V. Knighten
Changes of cardiac output caused by aging: an impedance cardiographic study. Angiology 31 (2): 75-81, 1980.

Madden, R.E., D. Agostino, L. Gyure
Some unusual aspects of microsphere migration at the microcirculatory level. Arch. Surg. 101: 425-528, 1970.

Madden, R.E., A. Paparo, M. Schwartz
Limiting vascular diameters in various organs. Arch. Surg. 96: 130-137, 1968.

Malik, A.B., J.E. Kaplan and T.M. Saba
Reference sample method for cardiac output and regional blood flow for determination in the rat. J. Appl. Physiol. 40 (3): 472-475, 1976.

Malik, A.B., D.J. Loegering, T.M. Saba and J.E. Kaplan
Cardiac output and regional blood flow following trauma. Circ. Shock 5: 73-84, 1978.

Marcus, M.L., D.W. Busija, C.J. Bischof and D.D. Heistad Methods for measurement of cerebral blood flow. Fed. Proc. 40: 2306-2310, 1981.

Marcus, M.L., D.D. Heistad, J.C. Ehrhardt and F.M. Abboud Total and regional cerebral blood flow measurement with 7-10, 15-, 25- and 50- um microspheres. J. Appl. Physiol. 40 (4): 501-507, 1976.

Maxwell, L.C., A.P. Shepherd, G.L. Riedel and M.D. Morris Effect of microsphere size on apparent intramural distribution of intestinal blood flow. Am. J. Physiol. 241 (Heart Circ. Physiol. 10): H408-H414, 1981.

McDevitt, D.G. and A.S. Nies
Simultaneous measurement of cardiac output and its distribution with microspheres in the rat. Cardiovasc. Res. 10: 494-498, 1976.

Mendell, P.L. and N.K. Hollenberg
Cardiac output distribution in the rat: comparison of

rubidium and microsphere methods. Am. J. Physiol. 221 (6): 1617-1620, 1971.

Meyers, F.H., E. Jawetz and A. Goldfien
Review of Medical Pharmacology (6th ed), Lang Medical Publications, Los Altos, California, 1978.

Montoye, H.J., P.W. Willis and D.A. Cunningham
Heart rate response to submaximal exercise: Relation to age and sex. J. Gerontol. 23: 127-133, 1968.

Moore, C.D., B.L. Gewertz, H.T. Wheeler and W.J. Fry
An additional source of error in microsphere measurement of regional blood flow. Microvasc. Res. 21: 377-383, 1981.

Morfaux, C. et J. Bralet
Etude des circulations regionales chez le rat par utilisation de microspheres. Influence de la noradrenaline, de l'angiotensine et de la vasopressine. J. Pharmacol (Paris) 8 (3): 261-268, 1977.

Morvai, V. and Gy. Ungvary
Effect of chronic exposure to alcohol on the circulation of rats of different ages. Acta Physiol. Acad. Sci. Hung. 53 (4): 433-441, 1979.

Moustafa, H.f and J.W. Hopewell
Age related changes in cardiac output, cephalic and cerebral blood flow in the rat. Int. J. Appl. Rad. Isotop. 32: 309-312, 1981.

Ohata, M., U. Sundaram, W.R. Fredericks, E.D. London and S. Rapoport Regional cerebral blood flow during development and aging of the rat brain. Brain 104: 319-332, 1981.

Papper, S.
Clinical Nephrology (2nd ed), Little, Brown and Company (Inc.), Boston, 1978, pp80.

Park, M.K., A.M. Diebl and J.M. Sunderson
Maturation of beta adrenergic receptor activity of rabbit aorta and pulmonary artery. Life Sci. 19: 321-328, 1976.

Peeters, L.H., G. Grutters and C.B. Martin Jr.
Distribution of cardiac output in the unstressed pregnant guinea pig. Am. J. Obstet. Gynecol. 138: 1177-1184, 1980.

Phibbs, R.H. and L. Dong
Nonuniform distribution of microspheres in blood

flowing through a medium sized artery. Can. J. Physiol. Pharmacol. 48: 415-421, 1970.

Popovic, V.P. and K.M. Kent
120-Day study of cardiac output in unanesthetized rats. Am. J. Physiol. 207 (4): 767-770, 1964.

Prange, A.J.Jr., J.E. White, M.A. Lipton and A.M. Kinkead
Influence of age on monoamine oxidase and catechol-o-methyl-transferase in rat tissue. Life Sci. 6: 581-586, 1967.

Prinzmetal, M., E.M. Ornitz, B. Simkin and H.C. Bergman
Arterio-venous anastomoses in liver, spleen and lungs. Am. J. Physiol. 152 (1): 48-52, 1948.

Prys-Roberts, C. (ed.)
The Circulation in Anaesthesia, Applied Physiology and Pharmacology, Blackwell Scientific Publications, Oxford, 1980.

Rakusan, K.
Postnatal Development of the Heart (in Hearts and Heart-like Organs. vol 1, G.H. Bourne ed.), Academic Press, Toronto, 1980.

Rakusan, K. and J. Blahitka
Cardiac output distribution in rats measured by injection of radioactive microspheres via cardiac puncture. Can. J. Physiol. Pharmacol. 52: 230-235, 1974.

Rakusan, K. and H. Marcinek
Postnatal development of the cardiac output distribution in rat. Biol. Neonate 22: 58-63, 1973.

Rauch, J.C. and D.D. Beatty
Sodium pentobarbital induced alterations in regional distribution of blood in Peromyscus maniculatus (deer mouse) and Eplesicus fuscus (big brown bat). Can J. Zool. 65: 1931-1935, 1977.

Richardson, P.D.I. and P.G. Witherington
Liver blood flow 1 Intrinsic and nervous control of liver blood flow. Gerontology 81: 159-173, 1981.

Roberts, J. and P.B. Goldberg
Changes in responsiveness of the heart to drugs during aging. Fed. Proc. 38: 1927-1932, 1979.

Rosenblum, R., W.D. Berkowitz and D. Lawson
Effect of acute intravenous administration of isoproterenol on cardiorenal hemodynamics in Man.

Circulation 38: 158-168, 1968.

Roth, G.S.
Hormone receptor changes during adulthood and senescence: significance for aging research. Fed. Proc. 38: 1910-1914, 1979.

Roth, J.A., A.J. Greenfield, S. Kaihara et al.
Total and regional cerebral blood flow in unanesthetized dogs. Am. J. Physiol. 219: 96-101, 1970.

Rothbaum, D.A., D.J. Shaw, C.S. Angell and N.W. Shock
Cardiac performance in the unanesthetized senescent male rat. J. Gerontol. 28 (3): 287-292, 1973.

Age differences in the baroreceptor response of rats. J. Gerontol. 29 (5): 488-492, 1974.

Rubanyi, G. and A.G.B. Kovach
Effect of pentobarbital anesthesia on contractile performance and oxygen consumption of perfused rat heart. Circ. Shock 7: 121-127, 1980.

Rudolph, A.M. and M.A. Heymann
The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. Circ. Res. 21: 163-184, 1967.

Sapirstein, L.A.
Regional blood flow by fractional distribution of indicators. Am. J. Physiol. 193 (1): 161-168, 1958.

Sasaki, Y. and H.N. Wagner Jr.
Measurement of the distribution of cardiac output in unanesthetized rats. J. Appl. Physiol. 30 (6): 879-884, 1971.

Sato, I., Y. Hasegawa, N. Takahashi, Y. Hurata, K. Shimimura and K. Hotta Age related changes of cardiac control function in man with special reference to heart rate control at rest and during exercise. J. Gerontol. 36 (5): 567-572, 1981.

Schlettwein-Gsell, D
Survival curves of an old age rat colony. Gerontologica 16: 111-115, 1970.

Schocken, D.D. and G.S. Roth
Reduced beta adrenergic receptor concentrations in ageing man. Nature 267: 856-857, 1977.

Schrier, R.W.
Effects of adrenergic nervous system and catecholamines on systemic and renal hemodynamics, sodium and water excretion and renin secretion. Kidney Int. 6: 291-306, 1974..

Schroeder, J.S., S.C. Robison, H.A. Miller and D.C. Harrison Effects of respiratory acidosis on circulatory response to isoproterenol. Am. J. Physiol 218 (2): 448-452, 1970.

Shanks, R.G.
The effect of propanolol on the cardiovascular responses to isoprenaline, adrenaline and noradrenaline in the anesthetized dog. Br. J. Pharmacol. 26: 322-333, 1966.

Simmes, H. S. and B.H. Berg
Longevity and the onset of lesions in male rats. J. Gerontol. 12: 244-252, 1957.

Snedecor, G.W. and W.G. Cochran
Statistical Methods (6th ed), Iowa State University Press, Ames Iowa, U.S.A. 1967.

Stanton, H.C., G. Brenner and E.D. Mayfield
Studies on isoproterenol-induced cardiomegaly in rats. Am. Heart J. 77 (1): 72-80, 1969.

Stark, K.
Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem. Pharmacol. 77: 1-124, 1977.

Stjarne, L. and J. Brundin
Beta 2- adrenoceptors facilitating noradrenaline secretion from human vasoconstrictor nerves. Acta Physiol. Scand. 97: 88-93, 1976.

Strandell, T.
Heart rate, arterial lactate concentration and oxygen uptake during exercise in old men compared with young men. Acta Physiol. Scand. 60: 197-216, 1964.

Stulcova, B.
Postnatal development of cardiac output distribution measured by radioactive microspheres in rats. Biol. Neonate 32: 119-124, 1977.

Tadepalli, A.S., G.M. Walsh and A.J. Tobia
Normal cardiac output in the conscious young spontaneously hypertensive rat: Evidence for higher oxygen utilization. Life Sci. 15: 1103-1114, 1974.

Tancredi, R.G., T. Yipintsoi and J.B. Bassingthwaighte
Capillary and cell wall permeability to potassium in isolated dog hearts. Am. J. Physiol. 229: 537-544, 1975.

Thormann, J., M. Gottwik, M. Schlepper and F. Hehrlein
Hemodynamic alterations induced by isoproterenol and pacing after aortic valve replacement with the Bjork-Shiley or St. Jude medical prosthesis. Circulation 63 (4): 895:904, 1981.

Tolnai, S. and B. Korecky
Lysosomal hydrolases in the heterotopically isotransplanted heart undergoing atropy. J. Mol. Cell. Cardiol. 12: 869-890, 1980.

Tripp, M.R., M.W. Meyer, S. Einzig, J.J. Leonard, C.R. Swayze and I.J. Fox Simultaneous regional myocardial blood flows by tritiated water and microspheres. Am. J. Physiol. 232 (2): H123-H190, 1977.

Tsuchiya, M., G.M. Walsh and E.D. Frohlich
Systemic hemodynamic effects of microspheres in conscious rats. Am. J. Physiol. 233: H617-H612, 1977.

Tuttle, R.S.
Age related changes in the sensitivity of rat aortic strips to norepinephrine and associated chemical and structural alterations. J. Gerontol. 21: 510-516, 1966.

van Brummelen, P., F.R. Buhler, W. Kiowski and F.W. Amann Age-related decrease in cardiac and peripheral responsiveness to isoprenaline: studies in normal subjects. Clin. Sci. 60: 571-577, 1981.

Vizek, M. and I. Albrecht
Development of cardiac output in male rats. Physiol. bohemoslov. 22 (6): 573-580, 1973.

Voorhees, A.B., H.J. Baker and E.J. Pulaski
Reactions of albino rats to injections of dextran. Fed. Proc. 76: 254-256, 1951.

Wallenstein, S., C.L. Zucker and J.L. Fleiss
Some statistical methods useful in circulation research. Circ. Res. 47: 1-9, 1980.

Warren, D.J. and J.G.G. Ledingham
Measurement of cardiac output distribution using microspheres. Some practical and theoretical

considerations. Cardiovasc. Res. 8: 570-581, 1974.

Weisfeldt, M.L., J.R. Wright, D.P. Shreiner, E Lakatta and N.W. Shock Coronary flow and oxygen extraction in the perfused heart of senescent male rats. J. Appl. Physiol. 30 (1): 44-49, 1971.

Weiss, B., L. Greenberg and E. Cantor
Age related alterations in the development of adrenergic denervation supersensitivity. Fed. Proc. 38: 1915-1921, 1979.

Wexler, B.C.
Spontaneous arteriosclerosis in repeatedly bred male and female rats. J. Atheroscler. Res. 4: 57-80, 1964 a.

Spontaneous coronary arteriosclerosis in repeatedly bred male and female rats. Circ. Res. 14: 32-43, 1964 b.

Wicker, P. and R.C. Tarazi
Importance of injection site for coronary blood flow determination my microspheres in rats. Am. J. Physiol. 242: H94-H97, 1982.

Woods, J.R. Jr., A. Dandavino, C.R. Brinkman 111, B.Nuwayhid and N.S. Assali Cardiac output changes during neonatal growth. Am. J. Physiol. 234 (5): H520-H524, 1978.

Yarger, W.E, M.A. Boyd and N.W. Schrader
Evaluation of methods of measuring glomerular and nutrient blood flow in rat kidneys. Am. J. Physiol. 235 (5): H592-H600, 1978.

Yates, M.S. and C.R Hiley
The effects of age on cardiac output and its distribution in the rat. Experientia 35 (1): 78-79, 1979.

Yin, F.C., H.A. Spurgeon, H.L. Green, E.G. Lakatta, M.L. Weisfeldt Age associated decrease in heart rate response to isoproterenol in dogs. Mech. Ageing Dev. 10: 17-25, 1979.

Yin, F.C., H.A. Spurgeon, G.S. Raizes, H.L. Green, M.L. Weisfeldt and N.W. Shock Age associated decrease in chronotropic response to isoproterenol. Circulation 54 (4): II-167, 1976.

Yin, F.C., H.A. Spurgeon, K. Rakusan, M.L. Weisfeldt and

E.G. Lakatta Use of tibial length to quantify cardiac hypertrophy: applications in the aging rat.
Am. J. Physiol 243 (Heart Circ. Physiol. 12): H941-H947, 1982.

APPENDIX

Table 13
Organ Weights in Sprague Dawley Rats
of Three Age Groups
(in Grams)

Organ	5 months n=7	12 months n=7	24 months n=7	
Right Ventricle	0.33 ± 0.02	0.32 ± 0.03	0.31 ± 0.03	
Left Ventricle	1.30 ± 0.04	1.40 ± 0.19	1.39 ± 0.06	
Total Heart	1.62 ± 0.05	1.72 ± 0.21	1.70 ± 0.08	
Lungs	3.05 ± 0.56	4.18 ± 0.72	4.66 ± 0.90	
Diaphragm	1.57 ± 0.09	1.79 ± 0.19	1.64 ± 0.14	
Liver Art.	20.59 ± 1.78	25.99 ± 1.23	23.58 ± 1.74	
Spleen	1.18 ± 0.11	1.08 ± 0.17	1.33 ± 0.23	
Stomach	2.17 ± 0.20	2.81 ± 0.12	3.39 ± 0.21	@
Small Intestine	7.99 ± 0.79	6.13 ± 0.65	7.98 ± 0.83	
Large Intestine	4.52 ± 0.78	3.74 ± 0.51	4.05 ± 0.46	
Portal	15.85 ± 0.50	13.76 ± 1.36	16.74 ± 1.46	
Kidneys	4.62 ± 0.18	5.55 ± 0.27	5.80 ± 0.42	
Adrenals	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	
Testes	4.08 ± 0.10	4.10 ± 0.17	3.64 ± 0.14	
Brain	2.13 ± 0.06	2.40 ± 0.04	2.36 ± 0.06	
Gastrocnemius	3.34 ± 0.23	3.39 ± 0.22	2.47 ± 0.13	@@
Skin	151.8 ± 9.68	178.5 ± 15.6	211.7 ± 15.4	
Carcass	432.3 ± 15.4	448.9 ± 31.1	458.5 ± 23.6	

"@" means ($p<.05$), "@@" means ($p<.01$) in ANOVA between age groups in this table.
Expressed as mean wet weight ± standard error. Portal refers to combined Spleen, Stomach, Small and Large Intestine. Carcass is remaining tissue, mostly bone, muscle and fat.

Table 14
Organ Weights in Lewis f/Mai Rats
of Two Age Groups
(in Grams)

Organ	5 months n=7	10 months n=6	
Right Ventricle	0.14 ± 0.01	0.14 ± 0.02	
Left Ventricle	0.65 ± 0.02	0.74 ± 0.02	#
Total Heart	0.79 ± 0.03	0.88 ± 0.04	
Lungs	1.86 ± 0.47	1.77 ± 0.10	
Liver Art.	14.14 ± 0.62	18.69 ± 0.47	##
Spleen	0.74 ± 0.09	0.82 ± 0.03	
Stomach	2.08 ± 0.08	2.69 ± 0.05	##
Intestine	7.86 ± 0.25	8.65 ± 0.45	
Portal	9.94 ± 0.30	11.34 ± 0.44	##
Kidneys	3.42 ± 0.15	4.28 ± 0.07	##
Transplant R. Vent.	0.07 ± 0.02	0.20 ± 0.02	##
Transplant L. Vent.	0.31 ± 0.03	0.47 ± 0.01	##
Total Transplant	0.39 ± 0.04	0.66 ± 0.03	##
Brain	2.38 ± 0.03	2.56 ± 0.07	#
Gastrocnemius	2.47 ± 0.13	3.19 ± 0.08	##
Skin	96.15 ± 1.69	142.68 ± 1.88	##
Carcass	284.23 ± 7.04	386.13 ± 6.84	##

"#" means ($p<.05$), "##" means ($p<.01$) in unpaired t-test between age groups in this table.
Expressed as mean wet weight ± standard error. Portal refers to combined Spleen, Stomach and Intestine. Carcass is remaining tissue, mostly bone, muscle and fat.

Table 15
Distribution of Cardiac Output After Isoproterenol
% of C.O. in Three Age Groups

Organ	5 months n=7	12 months n=7	24 months n=7
Right Ventricle	1.10 ± 0.17 *	1.57 ± 0.37	0.75 ± 0.11
Left Ventricle	5.81 ± 0.66	6.45 ± 1.44	3.22 ± 0.79
Total Heart	6.91 ± 0.77	7.91 ± 1.78	3.97 ± 0.86
Lungs	1.26 ± 0.25	1.17 ± 0.26	1.52 ± 0.40
Diaphragm	0.39 ± 0.03	0.46 ± 0.05	0.55 ± 0.06
Liver Art.	1.55 ± 0.30	4.30 ± 0.85	4.87 ± 1.32 @ #
Spleen	1.56 ± 0.23	0.79 ± 0.13 **	0.80 ± 0.12 * @@ #
Stomach	0.86 ± 0.10	0.87 ± 0.15 *	0.71 ± 0.17
Small Intestine	5.78 ± 0.79	4.77 ± 1.28 **	5.32 ± 1.54
Large Intestine	1.93 ± 0.45	1.53 ± 0.29	1.92 ± 0.56
Portal	10.57 ± 1.32	7.96 ± 1.71 **	8.75 ± 2.07 *
Kidneys	14.19 ± 1.92	7.04 ± 1.65 **	7.73 ± 2.54 @
Adrenals	0.15 ± 0.03	0.21 ± 0.06	0.26 ± 0.07
Testes	0.35 ± 0.02	0.37 ± 0.03 *	0.44 ± 0.06
Brain	1.09 ± 0.12	0.99 ± 0.19	0.85 ± 0.16
Gastrocnemius	0.41 ± 0.08	0.39 ± 0.06	0.41 ± 0.03
Skin	12.40 ± 1.48	19.50 ± 2.54	25.68 ± 4.17 @ ##
Carcass	50.38 ± 2.40	49.40 ± 3.84*	43.93 ± 1.79

"*" means ($p<.05$), "**" means ($p<.01$) in paired t-test with controls in Table 2.
"@" means ($p<.05$), "@@" means ($p<.01$) in ANOVA between age groups in this table.
"#" means ($p<.05$), "##" means ($p<.01$) in linear regression. Expressed as % of C.O. ± standard error in animals treated with o.2 ug/min·kg isoproterenol for 3-5 minutes. Lung refers to bronchial fraction and A-V shunts. Liver Art. refers to hepatic artery fraction. Portal refers to combined Spleen, Stomach, Small and Large Intestine fraction. Carcass is remaining tissue, mostly bone, muscle and fat.

Table 16
Organ Blood Flows After Isoproterenol
As ml/min·g in Three Age Groups

Organ	5 months n=7	12 months n=7	24 months n=7
Right Ventricle	7.04 ± 1.47 *	10.58 ± 2.41 *	4.06 ± 0.39
Left Ventricle	9.15 ± 1.36 *	10.46 ± 3.38	4.06 ± 0.90
Total Heart	8.75 ± 1.36 *	10.21 ± 2.91	4.03 ± 0.79
Lungs	0.95 ± 0.20	0.65 ± 0.09	0.67 ± 0.19
Diaphragm	0.51 ± 0.06	0.63 ± 0.09	0.64 ± 0.11
Liver Art.	0.16 ± 0.03	0.40 ± 0.09	0.37 ± 0.09
Spleen	2.80 ± 0.51	1.70 ± 0.22	1.21 ± 0.19 @ ##
Stomach	0.85 ± 0.15	0.69 ± 0.11	0.38 ± 0.07 ##
Small Intestine	1.63 ± 0.43	1.87 ± 0.71 *	1.12 ± 0.24
Large Intestine	1.11 ± 0.17	0.91 ± 0.13	0.80 ± 0.20
Portal	1.31 ± 0.15	1.33 ± 0.35	0.89 ± 0.15
Kidneys	6.19 ± 0.95	2.85 ± 0.87 **	2.27 ± 0.58 @@ ##
Adrenals	2.76 ± 0.53	5.39 ± 1.89	4.63 ± 1.21
Testes	0.17 ± 0.02	0.22 ± 0.04	0.23 ± 0.05
Brain	1.01 ± 0.12	1.04 ± 0.36	0.61 ± 0.09
Gastrocnemius	0.28 ± 0.08	0.30 ± 0.08 *	0.31 ± 0.05
Skin	0.16 ± 0.02 *	0.31 ± 0.09	0.27 ± 0.11
Carcass	0.24 ± 0.03 *	0.28 ± 0.05	0.19 ± 0.05

"*" means ($p<.05$), "**" means ($p<.01$) in paired t-test with controls in Table 3.

"@" means ($p<.05$), "@@" means ($p<.01$) in ANOVA between age groups in this table.
"##" means ($p<.01$) in linear regression. Expressed as ml/min·g ± standard error in animals treated with 0.2 ug/kg/min isoproterenol for 3-5 minutes. Lung refers to bronchial flow and A-V shunts. Liver Art. refers to hepatic artery flow. Portal refers to combined Spleen, Stomach, Small and Large Intestine flow. Carcass is remaining tissue, mostly bone, muscle and fat.

Table 17
Distribution of Cardiac Output After Anesthesia
% of C.O. in Two Age Groups

Organ	5 months n=7		10 months n=6	
Right Ventricle	1.21 ± 0.16	*	0.77 ± 0.14	
Left Ventricle	4.73 ± 0.17	*	4.10 ± 0.55	
Total Heart	5.94 ± 0.29	*	4.87 ± 0.67	
Lungs	5.29 ± 0.90	*	2.34 ± 0.66	#
Liver Art.	10.31 ± 1.39	**	13.19 ± 1.52	**
Spleen	1.74 ± 0.52	*	1.18 ± 0.04	*
Stomach	1.59 ± 0.45		1.31 ± 0.27	
Intestine	13.18 ± 1.09	**	9.73 ± 0.66	#
Portal	14.77 ± 1.33	*	11.03 ± 0.66	
Kidneys	24.24 ± 1.37	**	28.29 ± 0.55	**
Transplant R. Vent.	0.16 ± 0.05		0.44 ± 0.06	##
Transplant L. Vent.	0.70 ± 0.05		1.11 ± 0.20	
Total Transplant	0.86 ± 0.08		1.55 ± 0.25	#
Brain	2.02 ± 0.17		1.90 ± 0.21	
Gastrocnemius	0.10 ± 0.02		0.12 ± 0.02	*
Skin	6.16 ± 0.46	*	6.64 ± 0.36	
Carcass	27.25 ± 1.59	**	27.70 ± 0.63	**

"*" means ($p<.05$), "**" means ($p<.01$) in paired t-test with controls in Table 8.
"#" means ($p<.05$), "##" means ($p<.01$) in unpaired t-test between age groups in this table. Expressed as % of C.O. ± standard error. in anesthetized animals (45 mg/kg sodium pentobarbital). Lung refers to bronchial fraction and A-V shunts. Liver Art. refers to hepatic artery fraction. Portal refers to combined Spleen, Stomach and Intestine fraction. Carcass is remaining tissue, mostly bone, muscle and fat.

Table 18
Organ Blood Flows After Anesthesia
As ml/min·g in Two Age Groups

Organ	5 months n=7		10 months n=6	
Right Ventricle	6.51 ± 0.97		3.92 ± 0.84	
Left Ventricle	5.58 ± 0.64		3.65 ± 0.51	#
Total Heart	5.76 ± 0.68		3.67 ± 0.54	#
Lungs	3.01 ± 0.62		1.08 ± 0.27	#
Liver Art.	0.63 ± 0.06	**	0.57 ± 0.07	**
Spleen	2.12 ± 0.38	*	1.16 ± 0.07	**#
Stomach	0.71 ± 0.22		0.39 ± 0.08	**
Intestine	1.61 ± 0.31		0.91 ± 0.11	*
Portal	1.42 ± 0.27		0.78 ± 0.07	*
Kidneys	6.59 ± 0.87		5.28 ± 0.17	**
Transplant R. Vent.	1.52 ± 0.40		1.47 ± 0.16	
Transplant L. Vent.	1.74 ± 0.21		1.53 ± 0.25	
Total Transplant	1.71 ± 0.20		1.52 ± 0.22	
Brain	0.75 ± 0.06		0.59 ± 0.05	*
Gastrocnemius	0.03 ± 0.01	*	0.03 ± 0.00	
Skin	0.06 ± 0.01		0.04 ± 0.00	**
Carcass	0.09 ± 0.01	**	0.06 ± 0.00	**#

"*" means ($p<.05$), "**" means ($p,.01$) in paired t-test with controls in table 9.
"#" means ($p<.05$) in unpaired t-test with age groups in this table. Expressed as ml/min·g ± standard error. in anesthetized animals (45 mg/kg soduim pentobarbital). Lung refers to bronchial flow and A→V shunts. Liver Art. refers to hepatic artery flow. Portal refers to combined Spleen, Stomach and Intestine flow. Carcass is remaining tissue, mostly bone, muscle and fat.

The window for the Ce-141 extended from 135 to 155 KeV

The window for the Cr-51 extended from 310 to 330 KeV

Counts caused by Cr-51 in the Ce window were between 5 and 7% of those in the Cr window. No overlaps of counts caused by Ce-141 occured in the Cr window.

Background accounted for approximately 300-350 counts per 50 minutes.

Typical sample counts were between 10^3 and 10^6 counts per 50 minutes, depending on the tissue, isotope and age of the isotope.