EVALUATION OF LEFT AND RIGHT VENTRICULAR PERFORMANCE DURING VOLUME OVERLOADING IN NORMAL RATS AND THOSE WITH EXPERIMENTAL CARDIOMEGALY.

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Acknowledgements

I wish to thank my supervisors, Dr. M. Beznak and Dr. K. Rakusan, for their inspiration and friendship throughout the two years I spent in Canada working on this project. I also wish to thank all the members of the Department of Physiology at Ottawa University, and in particular Dr. B. Korecky and Dr. G. Mainwood, for their advice and help in the preparation of this thesis. I would also like to thank Dr. F. Depoca and Dr. J. Taylor of the National Research Council, Ottawa, and Mdme. T. Fournier, of Ottawa University Medical School, for their encouragement and assistance during the experimental procedures.

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ABBREVIATIONS.

BW	Body Weight
CRD	Chronic Respiratory Disease
DP	Developed Pressure
EDP	End-diastolic Pressure
EDV	End-diastolic Volume
HW/BW	Heart Weight, Body Weight ratio
IU	Infusion Unit
LV	Left Ventricle
LVCO	Left Ventricular Cardiac Output
LVDP	Left Ventricular Developed Pressure
LVEDP	Left Ventricular End-diastolic Pressure
LVSP	Left Ventricular Systolic Pressure
LVW	Left Ventricular Weight
LVW/BW	Left Ventricular Weight, Body Weight Ratio
PMP	Post-infusion Measuring Period
PVP	Polyvinylpyrrolidone
RV	Right Ventricle
RVCO	Right Ventricular Cardiac Output
RVDP	Right Ventricular Developed Pressure
RVH	Right Ventricular Hypertrophy
RVSP	Right Ventricular Systolic Pressure
RVW	Right Ventricular Weight
RVW/BW	Right Ventricular Weight, Body Weight Ratio
RVW/LVW	Right Ventricular Weight, Left Ventricular Weight Ratio.

1. INTRODUCTION

1. INTRODUCTION

For a number of years Doctor Beznak and her colleagues in this laboratory have been evaluating the performance of the left ventricle of rats under various conditions, such as myocardial damage (caused by isoproterenol treatment), left ventricular hypertrophy (produced by aortic constriction), hyperthyroidism and hypophysectomy, before and after treatment with various hormones. One of the major methods used to evaluate the performance of the left ventricle, under these varying conditions, was its response to volume overloading with a solution of PVP (polyvinylpyrrolidone). During infusion of this solution the indices of cardiac output and cardiac work rose to maximum values which were considered to be a measure of the reserve force of the heart.

As the right ventricle copes more easily with a volume overload than the left, it was assumed that the left ventricle would fail before the right during this experiment and that it was the maximum work of the left ventricle which was being measured unaffected by right ventricular failure. As this assumption had never been proven under these specific conditions, experiments were undertaken to try and determine whether it was valid. This assumption was examined in normal rats as well as those with right or left ventricular hypertrophy.

The many different methods which can be used to create cardiomegaly in rats are discussed briefly in the Literary Review. From these, constriction of the ascending aorta was chosen to produce left ventricular hypertrophy and constriction of the pulmonary artery was chosen to produce right ventricular hypertrophy. In these methods the stimulus responsible for ventricular hypertrophy is isolated, effecting only the heart and not altering or damaging the other tissues of the body. In addition, if the degree of constriction is carefully controlled it will result in an adequate degree of ventricular hypertrophy, in a short period of time, without damaging the cardiac tissue.

The experiment used to evaluate the cardiac performance of the three groups of rats was based on one originally designed by Dr. Beznak. Basal cardiac output and pressure measurements were made with the rats anaesthetised and were repeated after the infusion of set volumes of a PVP solution. The cardiac output was measured by the direct Fick method, this and other methods which have been used to determine the cardiac output of the rat are described in the Literary Review, along with the results of previous determinations made by other workers. Cardiac work was then determined as the product of the cardiac output and the femoral artery pressure. Although this parameter could be used as an index of left ventricular performance it was obviously not capable of differentiating between the performance of the right and left ventricles and to have compared it with right ventricular work would have involved the almost impossible feat, in an animal as small as a rat, of catheterising the pulmonary artery, so alternative parameters for evaluating ventricular performance were sought.

The problem of evaluating cardiac performance is a difficult one and is described in general terms, with particular emphasis on the performance of the heart as a pump, in the Literary Review. As many of the parameters of cardiac performance can only be obtained by cardiac catheterisation, techniques for catheterising the right and left ventricles of the rat were developed. This enabled simultaneous comparisons of right and left ventricular end-diastolic pressures, systolic pressure and developed pressure; the changes in these values, as well as those of cardiac output and cardiac work, made possible the accurate assessment of the point of failure of each ventricle during volume overloading.

In the Method the procedures for producing right and left ventricular hypertrophy, the determination of cardiac output, the infusion experiment and the techniques for measuring the right and left intraventricular pressures are described in detail.

The procedures chosen for producing right and left ventricular hypertrophy both involve a thoracotomy, which demands healthy rats, a reliable method of administering positive pressure anaesthesia and delicate, exacting surgery. Previously published measurements of intracardiac pressures in the rat had been obtained by needle puncture via the diaphragm or in the open-chested animal. Both of these techniques were regarded as too inaccurate and unsuitable for this experiment, so techniques for intravascular catheterisation of right and left ventricles of the rat had to be developed.

The results of this study are then presented and, as this was the first accurate recording of the right and left intraventricular pressures of the rat, some pressure tracings are included. Finally these results are discussed and then summarised.

After the main experiment two preliminary trials were undertaken. In the first the feasibility of using rats with right and left ventricular hypertrophy (produced by the methods developed in this experiment) to study the electrocardiographic changes taking place during the development of ventricular hypertrophy was investigated. In the second the possibility of measuring the myocardial contractility of these rats, using only a catheter transducer system, was investigated.

2. LITERARY REVIEW

2.A METHODS OF PRODUCING CARDIOMEGALY IN LABORATORY RATS.

Cardiomegaly generally takes place by hypertrophy (an increase in size) of existing muscle fibres but hyperplasia (an increase in number) of muscle fibres, occurs as well in the rapidly growing rat heart in the first three weeks of life. The precise mechanism responsible for cardiomegaly is not known but a variety of different methods can be used to produce it. In any of these methods the younger the rat when the stimulus is applied, the greater will be the increase in heart weight.

Some of these methods result in decreased growth or loss of weight, for instance in hyperthyroidism, weight loss occurs as the increased food intake is not sufficient to meet the increased energy requirements of the high metabolic state. It has been shown that the weight of the rat's heart follows changes in body weight very closely, whether these changes are due to growth or starvation, so if the body weights of the experiemental and control groups differ, heart weight comparisons must be made between animals of the same body weight. (Walter and Addis 1939) (Beznak 1954). The heart weight of animals of identical sex, breed and body weight, normally lies within the limits of $\pm$ 20% so unless the increase in heart weight is greater than 20% a true cardiac hypertrophy has not occured. (Korecky et al. 1966).

Some methods were designed specifically to increase the weight of one side of the heart alone. Although increasing the work of one ventricle leads to its hypertrophy and, in some cases, failure, ultimately the opposite ventricle may also be affected. Failure of a hypertrophied left ventricle results in pulmonary hypertension, hypertrophy and even failure of the right ventricle. In clinical and experimental cases of cor pulmonale decreased performance and enlargement of the left ventricle may also occur, although the reasons for this are not entirely clear.

The methods of producing cardiomegaly in the rat may be categorised as follows:-

(1) Methods Which Produce a Chronic Pressure Overload.

(a) Methods Producing Systemic Hypertension Which Result in Enlargement of the Left Ventricle.

(i) Aortic Constriction

(ii) Systemic Hypertension of Renal Origin.

(b) Methods Producing Pulmonary Hypertension Which Result in Enlargement of the Right Ventricle.

(i) Pulmonary Artery Constriction.

(2) Methods Which Produce a Chronic Volume Overload.

(i) Anaemia

(ii) Hyperthyroidism

(iii) Physical Exercise.

These three methods all produce an increase in the cardiac output which affects both ventricles equally, so enlargement of the whole heart takes place.

(3) Methods Which Produce a Chronic Volume and Pressure Overload.

(i) Polycythaemia

(ii) Chronic Intermittent Hypoxic Hypoxia.

In these two methods the predominant stimulus responsible for cardiomegaly is pulmonary hypertension, which results in enlargement of the right ventricle while the concomitant increase in cardiac output is of minor importance and affects both sides of the heart equally.

(4) Methods in Which The Main Stimulus Responsible for Cardiomegaly is Unknown.

(i) Catecholamines.

(ii) Ligation of Coronary Arteries.

(1) Methods which Produce a Chronic Pressure Overload.

(a) Methods Producing Systemic Hypertension which Result in Enlargement of the Left Ventricle.

(i) Aortic Constriction

The ascending aorta can be constricted via a thoracotomy incision or through the thoracic inlet, both of these operations involve quite delicate surgery. However, once one of these techniques is mastered, it can be a reliable method of producing left ventricular hypertrophy (LVH) in a short time, it requires no follow-up treatment and has the added advantage that the stimulus only affects the left side of the heart and not the other tissues of the body.

The size of the constricting device must be carefully chosen, as too severe a constriction will cause death from left ventricular failure and one which is too slight will not produce LVH. The degree of constriction required is one which will produce a significant LVH and is associated with acceptable mortality rates (designated by Beznak (1964) as below 30 %). The cardiomegalies produced by this method in adult rats are usually of moderate proportions, ranging from 22 to 42 % (Hadju and Beznak 1947; Kerr et al. 1961; Nair et al. 1968; and Lipana and Fanburg 1970), higher percentage increases being associated with unacceptable mortality rates. The degree of hypertrophy produced by banding the ascending aorta of weanling rats was twice that produced in adult rats (Stewart and Lindsey 1967). After 9 weeks constriction, in 40 g rats, the HW/BW had increased by 65 %, the LV free wall/BW by 61 % and the septum/BW by 61 %.

Beznak devised an operation for constricting the abdominal aorta with a silver ring, placed just below the diaphragm. This produced quite acceptable increases in heart weight ranging from 25 to 40 % (Beznak 1964) and increases in left ventricular weight

Aortic Constriction (Cont'd):

ranging from 35 to 45%. (Beznak 1958) (Korecky et al. 1965). This has the advantage of being a much simpler and quicker procedure than constricting the ascending aorta but the disadvantage of reducing the blood supply to the lower portion of the body.

(ii) Systemic Hypertension of Renal Origin.

LVH due to chronic pressure loading also occurs when renal hypertension is created by a variety of experimental methods. An undesirable side effect of these methods is the kidney damage which they also produce.

Excessive doses of corticosteroids produce a hypertension which is mainly due to their salt (Na Cl) retaining properties. Daily injections of 2-methyl-9-fluorocortisol (a potent synthetic corticosteroid with predominantly mineralocorticoid activity) for 8 months resulted in a significant cardiac hypertrophy of 42%. (Bois et al. 1961). However, a more efficient treatment is the combination of unilateral nephrectomy, implantation of desoxycorticosterone pellets and salt loading. Using this regime Hall et al. (1953) reported a heart weight increase of 56% in 4 weeks, while Beznak (1969) produced an increase of 38 $\pm$ 5% in 2 weeks, 58 $\pm$ 5% in 4 weeks and 64 $\pm$ 5% in 6 weeks.

Various surgical techniques which interfere with the kidney or its blood supply, result in renal hypertension which may lead to cardiac hypertrophy Hall et al. (1953) found that constricting the left renal artery of rats weighing 98g produced a cardiomegaly of 34 % in 9 days. Rather (1949) performed a unilateral nephrectomy and constricted the remaining kidney slightly with a ligature; an acceptable degree of hypertrophy was seen 40 days later. Chanutin and Barksdale (1933) removed approximately 80 % of the total kidney tissue, in a two stage operation. However they found that the

average increase in heart weight, in animals killed 43 to 225 days postoperatively, was only 15.6 %, and, as this value falls within the limits of normal variation of the heart weight body weight ratio (HW/BW), it cannot be regarded as a true hypertrophy.

(b) Methods Producing Pulmonary Hypertension Which Result in Enlargement of the Right Ventricle

(i) Pulmonary Artery Constriction

Stewart and Lindsay (1967) were the first to use pulmonary artery constriction to produce right ventricular hypertrophy (RVH) in the rat. The mean weight of the rats at the time of operation was 40 g which had increased to 244 g when they were killed 9 weeks later. At autopsy they displayed signs typical of right heart failure and a cardiomegaly of 69 %, all parts of the heart were enlarged but the biggest increase was in the free wall of the right ventricle (RV) (free wall RV 149 %, free wall LV 38 % and septum 14 %).

Pulmonary artery constriction is a popular technique in larger experimental animals but rarely used in rats, as isolating and constricting the pulmonary artery is extremely difficult in such small animals. However the advantage of this technique is that the stimulus is isolated and effects only the right side of the heart, whereas in the other methods used to produce RVH in the rat (chronic intermittent hypoxic hypoxia and polycythaemia) the stimulus also affects the metabolism of the other tissues of the body.

(2) Methods Which Produce a Chronic Volume Overload

(i) Anaemia

The increase in cardiac output and, hence, increase in cardiac work is the main factor responsible for the cardiac hypertrophy found in anaemia. Beznak et al. (1969) reports that the cardiac output of the rat first increases when the haemoglobin level falls below 7 g% and that this is also the critical level at which signs of cardiac

hypertrophy first appear. The increase in cardiac output is due to the decreased viscosity of the blood, the vasodilation of the tissues poorly supplied with oxygen by the anaemic blood, both of which decrease the resistive load on the heart.

Korecky et al. (1964) and (Beznak (1969) both report heart weight increases of over 100 % in 3 months in rats which were weaned onto a low iron diet. This degree of cardiomegaly is one of the largest produced in rats by any experimental means. Two factors may be responsible for this. Firstly the stimulus was applied over the period of fastest growth in the rat's heart when cardiomegaly takes place by hyperplasia as well as hypertrophy of the muscle fibres. (Korecky and French 1967) (Neffgen 1969). As already mentioned the younger the animal when the stimulus is applied the greater the hypertrophy. This was clearly demonstrated by Korecky et al. (1966) who found that reducing the haemoglobin level of adult rats to 6 g% (by triweekly bleedings) and then maintaining them on a low iron diet only increased the heart weight by 30 %, compared to increases of over 100 % seen in rats weaned onto this diet. Secondly, the anaemia develops slowly, allowing circulatory changes enough time to cope with the slowly increasing load; with a sudden overload, such as arterial constriction, heart failure often supervenes before an adequate degree of hypertrophy is obtained.

(ii) Hyperthyroidism.

An increase in cardiac output is also the major factor contributing to the development of cardiomegaly in experimental thyrotoxicosis. When rats are given daily injections of thyroxine, tri iodothyronine or fed iodinated casein they develop hyperthyroidism which results in an increase in metabolic rate in all cells of the body and hence an increase in oxygen utilization, this produces a relative anoxia in the tissues and hence an increase in cardiac output.

In addition the thyroid hormone increases myocardial contractility. At high dose rates increased food intake is not sufficient to meet the increased energy requirements of the metabolic rate and the animal loses weight.

Workers using hyperthyroidism report the production of cardiomegalies of moderate proportions. Sandler and Wison (1959) produced a 40 % heart weight increase after injecting thyroine for 8 weeks, while Beznak (1969) reported a 38 % increase after similar treatment and a 60 % increase after using thyroxine at a higher dose rate for 7 weeks. Gemmill (1958) produced a 37 % increase in heart weight by adding 3,3,5,-tri iodothyronine to the drinking water for 7 weeks, while Van Liere (1969) obtained a 32 % increase after injecting this compound for 2 weeks. Proulx et al. (1966) found that feeding weanling rats a diet containing 0.1 % iodinated casein for 38 days resulted in a 50 % increase in heart weight.

(iii) Physical Exercise

Daily physical exercise for long periods results in an increase in the cardiac weight due to the increased cardiac output. This is a difficult and time consuming method of producing cardiomegaly as rats are generally reluctant to exercise and have to be carefully supervised throughout each daily exercise period. The degree of hypertrophy produced by forcing rats to run on an electric treadmill is low, 10-20 %, (Grimm 1963) Krames (1967) and that produced by forcing them to swim is only moderate, approximately 30 % in those showing an increase in heart weight. (Korecky et al. 1966) (Aldinger 1970). Generally speaking, unless the effect of exercise is to be studied, use of an alternative method of cardiomegaly production is preferable.

(3) Methods Which Produce a Chronic Volume and Pressure Overload

(i) Polycythaemia

Injections of cobalt are used to stimulate polycythaemia; how this happens is not certain but it seems likely that the cobalt produces respiratory inhibition in liver and skeletal muscle. (Swigart (1965)). As the polycythaemia develops the resistance of the pulmonary vascular system gradually increases and the right ventricular weight (RVW) slowly increases; this is more physiological than the abrupt rise in ventricular weight which takes place after arterial constriction. An increase in cardiac output also accompanies the polycythaemia but is of secondary importance.

Swigart (1965) used daily injections of cobalt to stimulate polycythaemia, producing a 30 % increase in the free wall of the RV in 9 weeks. Using the same technique Souhrada (1967) produced a 22 % increase in the ratio of right to left ventricular weight, (RVW/LVW) in 21 weeks.

(ii) Chronic Intermittent Hypoxic Hypoxia

Hypoxic hypoxia causes constriction of the pulmonary vascular bed, resulting in pulmonary hypertension which, in chronic hypoxic hypoxia, produces RVH. Chronic hypoxic hypoxia is also associated with medial hypertrophy of pulmonary arterioles, polycythaemia, hypervolemia and an increase in cardiac output which is responsible for placing a volume overload on both ventricles.

In a series of experiments, Van Liere and his associates, utilized the effects of chronic intermittent hypoxic hypoxia to produce cardiomegaly in rats. The rats were placed in decompression chamber for 8 hours a day for periods ranging from 24 to 28 days. The mean percentage increases in heart weight ranges from 19 to 39 %, those in RVW from 54 to 57 % and those in LVW from 27 to 41 % (Van Liere et al. 1961; Van Liere et al. 1965; Krames et al. 1967)

Swigart (1965) obtained a higher degree of RVH (65 % in males and 81.7% in females) by subjecting rats to reduced atmospheric pressure 23 hours a day for 9 weeks but, in contrast to the previous authors, they did not find any indication of LVH.

Sobel and Graboyes (1958) found that combing reduced atmospheric pressure with elevated environmental temperature enabled an accurate prediction of the amount of time needed to produce a given hypertrophy. For instance a 50% increase in heart weight would be produced in 135-65 g rats, by 4 hours exposure a day to an atmospheric pressure equivalent to 12,500 ± 1,000 feet at a temperature of 37 ± 1 C for 12 consecutive days. Of all the techniques used to produce cardiomegaly in the rat this is the only one in which the results can be so accurately predicted.

(4.) Methods in Which the Main Stimulus Responsible for Cadiomegaly is Unknown.

(i) Catecholamines.

Injections of catecholamines, such as adrenaline, noradrenaline and isopropylnoradrenaline (isoprenaline) can be used to produce cardiomegaly but are not often used for this purpose. Rakusan et al. (1965) observed an increase in heart weight of 59 % after injecting isoprenaline for 15 days.

(ii) Ligation of Coronary Arteries.

Ligation of coronary arteries is not a very satisfactory method of producing cardiomegaly, as the percentage increase in heart weight is low (20 % Norman and Coers 1960) and inconstant, frequently falling within the limits of normal variation. (Korecky et al. 1966). The resultant myocardial damage renders the tissue unsuitable for biochemical and functional determinations, unless the changes taking place after coronary artery occlusion are to be studied.

2.B CARDIAC OUTPUT DETERMINATIONS IN LABORATORY RATS.

(1) Methods Used to Measure Cardiac Output in Rats

Cardiac output determinations have been performed in rats using the following methods:-

(a) Direct Fick Method.

(b) Dye - Dilution Technique.

(c) Radioisotope Dilution Technique.

(d) Thermodilution Technique.

(a) Direct Fick Method

In 1870 Fick suggested that the cardiac output could be calculated from the formula,

$$\text{Cardiac output (ml/min)} = \frac{\text{oxygen consumption of the body (ml/min)}}{\text{difference in oxygen content of arterial and mixed venous blood (ml/100ml).}} \times 100$$

The arterial blood sample may be drawn from any artery but to ensure proper mixing of the venous blood the venous sample must be drawn from the right side of the heart, by cardiac catheterisation, and preferably from the pulmonary artery.

Calculation of cardiac output by the direct Fick Principle is most accurate if the body is in a "steady state" i.e. a constant physiological condition, in which the alveolar and blood gas tensions, oxygen uptake and respiratory quotient are all constant. The volume of oxygen taken up in the lungs must equal the volume used in the tissues - serious errors result from storage or liberation of gas in or from the body, including the lungs. The oxygen uptake can only be measured at the mouth and does not always reflect the volume used in the tissues. However, if the patient is resting and calm for at least 10 to 15 minutes prior to measurement, the oxygen collected over a period of several minutes and any collection made during irregular respiration discarded, inaccuracies due to changing physiological conditions should be reduced to a minimum.

Since the oxygen consumption used in the direct Fick method is the mean value for the gas collection period, it is necessary to obtain the arterio-venous difference for the same period of time. This value should be a mean in relation to the blood flow, the volume of blood sampled being in relation to the volume of blood passing the sampling point - this is a volume average mean. In practice a time average sample is obtained, the sample is drawn into a syringe at a constant rate over the sampling period and no attempt is made to weight the portion of the sample taken when the flow is fast as compared to the portion taken when the flow is slow. If either the arterio-venous difference or the flow past the sampling site remain constant over the sampling period, the volume average equals the time average, but if both are changing, this is not true.

It was originally assumed that utilization of oxygen in the pulmonary circulation would be so small that it could be overlooked. This assumption was queried for a number of years, but in 1955 Mitchell and Cournard showed that the lactic acid production of the lungs is small and hence lung metabolism is a negligible source of oxygen utilization. (Mitchell and Cournard 1955).

The bronchial flow is not included in cardiac output measurements made by the direct Fick Principle, normally this is unimportant, only amounting to 1-3 % of the total cardiac output, but it is increased and may assume importance in certain pulmonary diseases.

(b) Dye-Dilution Techniques.

In this technique a known amount of dye or indicator, which does not diffuse out of the circulation, is injected into the venous circulation and its concentration continuously recorded at a peripheral artery. The injected substance begins to appear after a delay of 6-15 seconds, it then builds up to a peak concentration and rounds off.

When the log concentration is plotted against time the descending limb can be extrapolated to a minimum value, but it never actually reaches this value as recirculation commences as the limb is still descending. The time of passage of the dye on its first circulation is the time between its injection and the instant it has reached its minimum value (as obtained by extrapolation of its descending limb). The cardiac output is then calculated from the formula,

$$\text{Cardiac Output (L/min)} = \frac{\text{Amount of indicator injected. (mg)}}{\text{Mean concentration of indicator for first circulation (mg/L)} \times \text{time for first circulation (sec).}} \times 60$$

Dyes such as indocyanine green, Evans blue and Coomassie blue are commonly used as indicators, they may be injected into the blood stream as a bolus (instantaneously) or continuously at a constant rate - the former is most widely used.

(c) Radioisotope Dilution Technique

Radioactive substances, such as T-1824, radioactive iodinated albumin and radioactive red blood cells, can be used instead of dye in the above method. When radioactive substances are used, a Geiger counter or scintillation counter is used to measure the concentration curves in the arterial tree.

(d) Thermodilution Technique

In this technique cold saline or blood is injected instead of dye and the temperature of the blood measured at a peripheral artery.

CARDIAC OUTPUT VALUES IN NORMAL RATS - TABLE 1

Investigator	Body Weight in g	Sex	Anaesthetic	Technique	Cardiac Output ml/min/Kg
Takacs and Vajdu (1963)	150-166	M	Pentobarbital Na 40 mg/Kg	Dye-dilution	280 ± 105
Takacs (1965)	160-180	M	Pentobarbital Na 40 mg/Kg	Dye-dilution	293 ± 45
Blood et al. (1950)	180-200	?	Pentobarbital Na 37.5 mg/Kg	Fick Principle	245
Beznak (1958)	160-220	M	Pentobarbital Na 40 mg/Kg	Fick Principle	258
Mandel and Sapirstein (1962)	190-250	F	Pentobarbital Na 40 mg/Kg	Radioisotope dilution	278 ± 73
Vidt et al. (1959)	180-230	F	Pentobarbital Na 40 mg/Kg	Radioisotope dilution	230 ± 41
Sapirstein et al. (1960)	175-225	?	Pentobarbital Na 40 mg/Kg	Radioisotope dilution	231 ± 43
Szabo et al. (1966)	131-255	M	Pentobarbital Na 50 mg/Kg	Dye-dilution	220
Bencsath and Takacs (1966)	160-250	M	Pentobarbital Na 40 mg/Kg	Dye-dilution	252
Sapirstein (1958)	225-275	F	Pentobarbital Na 40 mg/Kg	Radioisotope dilution	205

CARDIAC OUTPUT VALUES IN NORMAL RATS - TABLE 1 (Cont'd)

Investigator	Body Weight in g	Sex	Anaesthetic	Technique	Cardiac Output ml/Kg
Popovic and Kent (1964)	305-345	M	Pentobarbital Na 40 mg/Kg	Fick Principle	204 ± 18
Hoelscher (1954)	?	?	Pentobarbital sodium 30 mg/Kg	Fick Principle	287 ± 70
Reininger and Sapirstein (1957)	?	?	Pentobarbital sodium 40 mg/Kg	Radioisotope-dilution	223 ± 59
Mandel and Sapirstein (1962)	?	F	Pentobarbital sodium 40 mg/Kg	Radioisotope-dilution	231 ± 43
Dawson et al. (1968)	317-477	M	Pentobarbital sodium 20 mg/Kg	Thermodilution	255 ± 42
Richardson et al. (1962)	350-550	?	Pentobarbital sodium 24 mg/Kg	Thermodilution	238 (196-299)
Vidt et al. (1959)	180-230	F	Ether, stage 2-3.	Radioisotope-dilution	350 ± 36
Bullard (1956)	250-350	M	Ether	Dye-dilution	210 ± 45
Kallay and Takacs (1961)	?	M	Urethane 1g/Kg IP	Dye-dilution	244
	?	M	Choralase 0.1g/KgIV	Dye-dilution	328

CARDIAC OUTPUT VALUES IN NORMAL RATS - TABLE 1 (Cont'd)

Investigator	Body Weight in g	Sex	Anaesthetic	Technique	Cardiac Output ml/min/Kg
Szabo et al. (1966)	131-255	M	Awake, restrained	Dye-dilution	420
Becath and Takacs (1966)	160-250	?	Awake, restrained	Dye-dilution	99
Stevens (1969)	215-250	F	Awake, unrestrained	Dye-dilution	305
Popovic et al. (1969)	243	F	Awake, unrestrained	Fick Principle	398 ± 41
Jansky and Hart (1968)	355	?	Awake, unrestrained	Fick Principle	284
Popovic and Kent (1964)	300-500	M	Awake, unrestrained	Fick Principle	286 ± 25
Dawson et al. (1968)	317-477	M	Awake, unrestrained	Thermodilution	320 ± 12

mean ± standard deviation

? information not given.

This technique has the advantage of eliminating recirculation, as by the time the blood has recirculated it has regained body temperature, but the disadvantage that the cold may leak from the blood vessels between the point of injection and the point of sampling.

(2) A Survey of the Results of Cardiac Output Determinations in Normal Rats

(a) Introduction

The results of cardiac output determinations in normal rats are influenced by three main factors:-

(i) The age of the rat, usually reflected by its weight.

(ii) The rat's state of consciousness.

(iii) The method used to perform the determination.

In man, cardiac output is usually expressed per unit of body surface, i.e. as cardiac index, for it is generally accepted that the cardiac output is closely related to body size but the nature of this relationship has never been satisfactorily determined and has been challenged by those who feel a more reliable index could be obtained using multiple regression coefficients between cardiac output, age, heart rate and body size. This idea has not won approval for it is felt that it makes so little difference in practice that the cardiac index continues to be used. (Wade and Bishop 1962).

Although a few authors express the cardiac output of rats per unit body surface, the majority use only, or give also, the cardiac output per unit body weight and the latter is the value which will be discussed here.

From a series of measurements of human cardiac outputs at various ages, which had been collected from a number of sources, Wade and Bishop concluded that, "it seems likely that the cardiac index is at its largest in the child and young adolescent and then decreases throughout life". Despite the fact that this has not been reported in the rat a similar pattern emerges when looking at the results of different workers; the cardiac output per Kg body weight increases until maturity is reached (at approximately 100-50 g) and then decreases slowly with age and hence increasing weight.

Many comparative studies have been made to try and determine if there is any difference between the results of cardiac output determinations, of normal humans, measured by the direct Fick Method and Indication Dilution Methods (using dye or radioisotope as indicator). An interesting and well established fact is that 25 % of these results disagree. This lack of agreement is mostly due to the poor repeatability of the two methods and, despite lack of agreement in individual subjects, there is little systematic difference between these two methods (Wade and Bishop 1962). This also seems to be true of cardiac output determinations in the rat, using these three techniques, however those results which were obtained by the thermo-dilution technique have all been higher than those determined by other methods.

(b) Relationship Between Age and Cardiac Output in the Rat.

Considering the cardiac outputs of rats under Pentobarbital sodium anaesthesia (30-40 mg/kg), which were determined by either the direct Fick Method or the Dye-Dilution Method or Radioisotope

Dilution Technique, it can be seen that the cardiac output per Kg body weight is high in the young rat and slowly decreases with age. The cardiac output decreases from values of approximately 290 ml/min/Kg in 105 g rats (Takas and Vajdu (1963) and Takas (1965)) to values ranging from 220 to 258 ml/min/Kg in 200 g rats (Blood et al. (1950)) Beznak (1958) Vidt et al. (1959) Sapirstein et al. (1958) and Popovic and Kent (1964). Similarly, in unanaesthetised rats the cardiac output per Kg is higher in younger rats (Szabo et al. (1966) Bencsath and Takacs (1966)) than older, heavier ones (Popovic and Kent (1964) and Jansky and Hart (1968)). However, this relationship may be obscured by the effects of different types or degrees of anaesthesia, by varying degrees of stress in unanaesthetised rats and by systematic differences between methods.

(c) Methods Used to Determine Cardiac Output of Normal Rat

(i) Direct Fick Method

One of the earliest determinations in the rat was made in 1950 by Blood et al. Using the direct Fick Method with the rat under Pentobarbital sodium anaesthesia, they obtained a value of 245 ml/Kg/min in rats weighing 180-200 g. Similar results were obtained in the next few years by Hoelscher 1954 and Beznak (1958) (1959) using the same method and anaesthesia. Ideally, the venous samples used in these determinations should be obtained from the pulmonary artery, but this would have been extremely difficult in animals as small as rats. Blood et al. drew the venous sample from the right atrium but both Hoelscher and Beznak tried to catheterise the right ventricle - passing a polyethylene catheter down the right jugular, through the right atrium and into the ventricle, using as a guide, measurements of the distance from the external jugular to the right ventricle previously obtained on corpses. The position of the catheter was verified at autopsy, but this method was not completely successful and Beznak (1959) reports that in the majority of cases the catheter was found

in the right atrium; although any determination made when the catheter was found to have been in the superior vena cava or inferior vena cava were discarded, values from either the right atrium or right ventricle were used.

Later, Popovic et al. (1963) developed a more reliable method for catheterising the right ventricle in which a narrow bore polyethylene tube was manipulated into the right ventricle with the aid of a removable guide wire. This method was utilized by three groups of workers (Popovic and Kent 1964, Jansky and Hart 1968 and Popovic et al. 1969) who measured the cardiac output of unanaesthetised rats using the direct Fick Method, obtaining blood samples from cannulae chronically implanted in the right ventricle and aorta.

(ii) Radioisotope Dilution Technique

Using the Radioisotope Dilution Technique on rats under Pentobarbital sodium anaesthesia, most workers obtained results of approximately 230 ml/Kg/min, in 200 g rats. Mandel and Sapirstein (1962) were surprised to find a higher rate, 278 ml/Kg/min, in a group of 17 rats (weighing 190-250 g), as the average in his colony in the last two years had been 231 ml/Kg/min.

(iii) Dye Dilution Technique

Utilizing the Dye Dilution Technique, with Evans blue as indicator and the rat under Pentobarbital sodium anaesthesia, Szabo et al. (1966) and Bencsath and Takacs (1966) reported similar results to those obtained by the previous two methods. Takacs and Vajdu (1963), Roger and Menyhart (1967) and Takacs (1965) all reported higher cardiac outputs but this was to be expected as their rats were lighter and correspondingly younger than those previously discussed. The dye dilution technique was also employed by other workers when examining anaesthetic agents other than Pentobarbital sodium (Bullard 1956, Kallay and Takacs 1961) and when performing determinations on unanaesthetised but restrained rats (Szabo et al. 1966 and Bencsath and Takacs 1966).

(iv) Thermodilution

Cardiac output determinations using the Thermodilution technique in anaesthetised and unanaesthetised rats have all yielded higher results than those obtained by other methods. The values obtained by Dawson et al. (1968) and Richardson et al. (1962) using the thermodilution technique in 350-500 g rats, under Pentobarbital sodium anaesthesia, were close to those obtained in 200 g rats by other methods and therefore higher than expected for these older rats. Weeks and Csordas (1963) reported that cardiac outputs obtained by the thermodilution technique were consistently higher than those obtained by the Fick Method in unanaesthetised rats - but no values were given. Using the Thermodilution Technique in unanaesthetised rats weighing 317-477 g, Dawson et al. (1968) obtained a cardiac output of 320 $\pm$ 12 ml/min/Kg higher than those obtained by Jansky and Hart (1968) and Popovic and Kent (1964) when using the Fick Principle in unanaesthetised rats of similar weights.

(d) Anaesthetics Used for Cardiac Output Determinations.

(i) Pentobarbital sodium

Most cardiac output determinations in anaesthetised rats have been performed using Pentobarbital sodium - the usual dose rate being 30-50 mg/Kg I P. Two workers utilizing lower dose rates (Dawson et al. 1968 and Richardson et al. 1962) obtained higher results than would have been expected from the weight of their rats but this may have been partly due to the method used, the thermodilution technique.

Although a popular anaesthetic for measuring cardiovascular parameters in experimental animals, Pentobarbital sodium anaesthesia itself will influence these parameters. The effect of Pentobarbital anaesthesia on the cardiovascular system of experimental animals has been studied by many workers. During 4 hours of anaesthesia in the dog, Olmsted and Page (1966) found a slow but continual rise in blood pressure, heart rate, cardiac output and peripheral resistance.

They attributed the increase in heart rate to a vagal blocking action of the Pentobarbital and the increase in blood pressure to either the increase in peripheral resistance or to the increase in cardiac output. Rushmer (1961) ascribed many of the changes seen with Pentobarbital Anaesthesia to depression and distortion of the cardioregulatory influences of higher and lower levels of the nervous system and suggested that the influence of some higher centres may be eliminated entirely. Carson (1965) thought that the variation in cardiac output, found in animals under Pentobarbital anaesthesia, was due to the difficulty of obtaining a steady state in regard to acid-base balance status. Spontaneous metabolic acidosis frequently develops, and worsens with time, resulting in depression of the cardiac output, conversely respiratory acidosis, from depression of respiration, elevates the cardiac output and the resultant value is influenced by both of these factors.

(ii) Anaesthetics Other than Pentobarbital sodium.

Three groups have tried using anaesthetics other than Pentobarbital sodium for cardiac output determinations in the rat. Vidt (1959) found that the cardiac output of 180-230 g rats measured under Pentobarbital anaesthesia was consistent with results obtained by other workers but measured under ether was one and a half times as great. With ether, the myocardial, cerebral and carcass blood flows were higher than with Pentobarbital but the reverse was true of the renal, splanchnic and cutaneous flows. Vidt urges caution when considering cardiovascular measurements made under anaesthesia.

Bullard (1956) also measured the cardiac output under ether anaesthesia; in rats weighing 250-350 g he obtained a cardiac output of 209 ml/min/Kg. This was consistent with results obtained in heavier rats by other workers (Sapirstein 1958 and Popovic and Kent 1964).

The discrepancy between these two results may have been due to the different stages of anaesthesia employed; Vidt kept his rats at stage two to three while Bullard let his rats breathe away most of the ether before measurements were made.

Kallay and Takacs (1961) decided that Pentobarbital was a more suitable anaesthetic for circulatory experiments in rats, than Urethane or Choralase as both these had deleterious effects on renal blood flow; Urethane resulted in marked hypotension, while Choralase increased the blood flow to the skin and produced a slight fall in blood pressure. The cardiac outputs were also quite different but as the rat's body weight was not given it is difficult to compare these results with those already discussed.

(e) Cardiac Output Determinations Performed in Unanaesthetised Rats.

The difficulty of interpreting results obtained under anaesthesia led to the development of techniques to measure the cardiac output of unanaesthetised rats. Some techniques involved restraint of the rat but in others it was possible to measure the cardiac output of the unrestrained rat. Cardiac output values from unanaesthetised rats are all higher than those from anaesthetised rats of the same body weight, this is partly due to the removal of the depressive effect of the anaesthetic and partly due to the stressful effect of the environment.

(i) Unanaesthetised and Unrestrained

Popovic and Kent (1964) were first to give a full account of the measurement of the resting, unrestrained and unanaesthetised rat. They used the direct Fick Method obtaining blood samples from cannulae chronically implanted in the right ventricle and aorta. Initially the cardiac output of 47 rats, weighing 305-345 g, was determined while the rats were still under Pentobarbital anaesthesia - it averaged 205 $\pm$ 18 ml/min/Kg. After recovering from the cannulation procedure

the cardiac output of each unanaesthetised rat was determined after the rat had been placed in a metabolism jar and allowed to settle for 3-4 hours. These determinations were repeated several times in the next 120 days - during this time the rat's weight increased from 320 to 498 g and the cardiac output averaged 286 ± 25 ml/min/Kg.

Jansky and Hart (1968) using the same technique as Popovic and Kent on unanaesthetised rats of similar weight, obtained nearly identical results, however, Dawson et al. 1968 using the Thermodilution Technique on resting unanaesthetised rats of identical weight to those used by Popovic and Kent (1964), obtained a somewhat higher result, but, as already mentioned, results obtained by this method in the rat are usually higher than those determined by the Fick Method.

Popovic repeated his experiments in 1969 using 243 g female rats and was surprised to find a much higher cardiac output, 398 ml/min/Kg, than in his previous experiments; he offered as explanation the different sex and lighter weight of these rats. However, Stevens (1969) utilizing the Radioisotope Technique Dilution Technique in female rats of similar weight (215-250 g) obtained a somewhat lower value (305 ml/min/Kg).

(ii) Unanaesthetised and Restrained

In lighter rats, Szabo et al. (1966) and Benosath and Takacs (1966) both obtained much higher results with unanaesthetised rats than with those under Pentobarbital sodium. The results obtained under Pentobarbital agree with those of other workers but it is difficult to compare the results of the unanaesthetised rats, as they were obtained under conditions of much greater stress than those of other workers - the rats were tied down while cannulation and cardiac output determinations were made under local anaesthesia.

(3) Cardiac Output Determinations in Rats with Experimental Cardiomegaly.

To my knowledge only one worker has reported cardiac output determinations in whole rats (as opposed to heart-lung preparations) with experimental cardiomegaly. Beznak found that the cardiac output of rats with LVH due to constriction of the abdominal aorta did not differ from that of the control animals. In one report the cardiac output of rats with a 45 % LVH was 50 $\pm$ 5 ml/min while that of control rats was 48 $\pm$ 4 ml/min. (Beznak 1958)

2.C PHYSIOLOGICAL PARAMETERS, OBTAINED BY CARDIAC CATHETERISATION, WHICH AID IN EVALUATION OF CARDIAC FUNCTION.

(1) Principle Factors Determining the Performance of the Intact Heart.

The performance of the intact heart is determined by three factors:-

(a) Initial fibre length, which corresponds to ventricular end-diastolic volume (EDV) or ventricular end-diastolic pressure (EDP). The equivalent of preload.

(b) The systolic intramyocardial tension during ejection. The equivalent of afterload.

(c) The contractility or contractile state of the myocardium.

It is also influenced by the synergy of contraction and the heart rate. An interplay amongst these factors, each of which in turn is influenced by a number of other factors (such as the sympathetic nervous system, blood volume etc), governs the performance of the intact heart.

(a) Initial Fibre Length

The initial fibre length (end-diastolic fibre length) of the ventricular muscle determines the point at which the ventricle operates along its function curve. The Frank-Starling or ventricular function curve of the ventricle (analogous to the length tension curve of the heart muscle) relates the end-diastolic fibre length (usually measured as the EDV or EDP) to the mechanical activity of the ventricle (measured as stroke volume, stroke work etc.). The normal ventricle usually operates along the ascending limb of the curve, on which increasing end-diastolic fibre lengths result in augmentation of mechanical activity until, at a critical fibre length, peak performance is attained. When the fibres are stretched beyond this critical length the ventricle operates along a plateau and then along the descending limb of the curve, on which increasing fibre lengths are associated with decreased mechanical activity. (Starling 1915).

The failing ventricle in the intact heart usually operates along the depressed ascending limb of a lowered flattened ventricular function curve, rather than along the ascending limb. (Katz 1965). Monroe et al. (1970) has shown that the intact isolated LV will only function along a descending limb at unphysiological EDPs of 60-100 mm Hg and above; although some workers have reported ventricles operating on descending limbs in cases of anaemia, coronary artery constriction, excessive myocardial oedema and inadequate perfusion pressure, this situation will only prevail for a short time as it is rapidly fatal.

In the normal heart, the Frank-Starling mechanism is operative but is rarely a dominant factor in determining ventricular performance: it is essential for preserving the balance between the pumping of the right and left ventricles and does so by providing immediate regulation of the stroke volume of each. (Hamilton 1955). However, in the diseased heart, which is using its hormonal and reflex mechanisms maximally, the Frank-Starling mechanism is a significant means of increasing performance.

Electron microscopic studies have recently revealed the ultra-structural basis of the Frank-Starling mechanism. (Sonnenblick et al. 1964). These studies have shown that the muscle fibres contain overlapping layers of contractile myofilaments, actin and myosin, which are arranged within a basic functional unit called a sarcomere. As the end-diastolic fibre length increases, less overlapping of actin filaments occurs at the centre of the sarcomere, this increases the number of contractile sites available between the actin and myosin resulting in quantitive increases in force generating reactions and the ventricle moves up the ascending limb of the ventricular function curve. At the apex of the curve the length of the sarcomeres are stretched to an optimum value which allows maximum interaction between

the actin and myosin. When sarcomeres are stretched beyond this point the myofilaments begin to disengage, less tension is produced and the ventricle operates along the plateau and eventually the descending limb of the curve. From correlating electron microscopic observations of the myocardium of dogs and cats with pressure-volume curves from the same ventricles, Spotnitz et al. 1966 concluded that the optimum sarcomere length is 2.25 ± 0.04 , which corresponds to an EDP of about 10.12 mm Hg in the LV (Spotnitz et al. 1966) and slightly less in the RV (Leyton et al. 1970).

The end-diastolic fibre length is influenced by atrial systole, total blood volume and the distribution of the blood volume as determined by body position, intrathoracic pressure, venous tone and the pumping of the skeletal muscles.

(b) Afterload

The afterload or systolic intramyocardial tension during ejection is established by instantaneous aortic pressure, the Laplace equation relating tension to the product of pressure and radius (volume) and wall thickness. (Mason et al. 1970).

(c) Contractility

The contractility (contractile or inotropic state) of the myocardium determines ventricular performance at a given preload and afterload, and is shown by the relative position of the ventricular function curve. It is uninfluenced by loading conditions but is altered by inotropic interventions such as circulating catecholamines, the sympathetic nervous system, rate and rhythm of cardiac contraction and injections of inotropic agents. An alteration in the contractile state implies a qualitative alteration in the rate of reaction of the sub-cellular contractile sites, between actin and myosin.

Contractility can be defined in the terms of muscle mechanics as the position of the force velocity curve - the curve expressing the inverse relationship between the velocity of myocardial shortening and the development of tension within the ventricular wall. An increase in contractility is displayed by an upward movement of the upper part of the curve, elevating the extrapolated value at the ordinate, i.e. the maximum velocity of shortening of the contractile element (V max) - similarly a decrease in contractility is associated with a downward movement of V max. Changes in preload alter the position of the lower portion of the curve, an increase moving the intercept on the abscissa (analogous to the peak of the ventricular function curve) to the right, but do not effect the position of V max, (Sonnenblock 1969) (Ross 1969).

Of all the factors determining ventricular performance, the fundamental abnormality in experimental and human congestive heart failure is depressed contractility. This is also true for idiopathic cardiomegalies, ventricular hypertrophy secondary to chronic pressure loading and coronary artery disease.

The synergy or temporal sequence of ventricular contraction normally takes place in a co-ordinated fashion resulting in an integrated inward movement of the wall, which contributes to the pumping of the ventricles. However electrical disturbances, such as left bundle branch block, or localized pathological lesions, such as coronary artery disease, produce an asynergy resulting in disorderly distribution of contraction, which contributes to cardiac dysfunction and failure. (Herman and Gorlin 1969).

Increases in heart rate are not an important factor in increasing the cardiac output in normal subjects at rest, but are responsible for some increase during exercise. However an increase in heart rate is always associated with an increase in the inotropic state and in subjects

with diseased hearts this factor is very important in maintaining or elevating the cardiac output.

(2) Clinical Evaluation of Cardiac Performance, Using Cardiac Catheterisation.

(a) Cardiac Pump Performance

(i) End-diastolic Volume and End-Diastolic Pressure

Reliable methods of measuring EDV are available but generally thought too complicated for routine use. EDP is an easier parameter to measure and is frequently used as a guide to EDV despite Starling's warning that "the diastolic volume may change without corresponding alteration in filling pressure," (Starling 1915).

The ventricular pressure-volume curve and ventricular function curve demonstrate that when EDP is normal, or slightly elevated, it is fairly insensitive as an indicator of EDV or in the assessment of ventricular function. The ventricular pressure volume curves are relatively flat when EDP is normal or slightly elevated so, for any level of diastolic compliance, large changes in EDV result in relatively small changes in EDP. The ventricular function curve rises steeply when the EDP is normal or slightly elevated, so large changes in myocardial contractility result in small changes in EDP. (Braunwald and Ross 1963).

Although elevation of EDP is frequently a sign of heart failure it may also be due to a number of other factors. If a volume load is placed on a normally functioning heart by a valvular defect, a circulatory shunt, an intravenous infusion, anaemia, beri beri or excessive retention of salt and water due to stercid medication or kidney disease, the ventricle may move up the ventricular function curve. The resultant rise in EDP does not necessarily indicate myocardial failure, despite the symptoms of circulatory congestion produced by some of these states. (Eichna 1966; Braunwald et al. 1957).

If the diastolic compliance (end-diastolic pressure-volume relationship) is reduced by such conditions as a concentric myocardial hypertrophy, pericardial constriction, cardiac tamponade, incomplete relaxation during tachycardia or an infiltrative process of the myocardium, an abnormally high EDP may be necessary to produce a normal EDV but this will not necessarily stretch the sarcomeres to a point where myocardial function will be impaired. (Sandler and Dodge 1963). Due to the close anatomical relationship of the two ventricles the compliance of either is decreased in proportion to the filling of the opposite one. When one ventricle is grossly distended, so that its EDV is abnormally elevated (e.g. selective failure of either ventricle), a degree of ventricular filling in the opposite ventricle which is normally associated with a normal EDP, now produces an elevated EDP. (Taylor et al. 1967). In cases like these where the EDP and EDV change independently, changes in sarcomere length (and therefore the Frank-Starling mechanism) parallel changes in EDV.

Neither is the EDP invariably above normal in cases of heart failure. In ventricular dilation without thickening of the myocardial wall, e.g. some patients with mitral insufficiency, there is an increase in diastolic compliance, so the EDP may remain within normal limits while the EDV is greatly increased. (Dodge et al. 1962). Some cases of heart failure have been reported in which the EDP was not elevated above the upper limits of the normal range, but was sufficiently high to cause cardiac dysfunction in these particular patients.

In addition to the problems associated with interpreting changes in EDP, inaccuracies of measurement are frequently encountered. These inaccuracies are usually due to the difficulty of positioning the external manometer in the closed chest patient or to referring EDP to atmospheric rather than intrapericardial or intrapleural pressure.

Providing the investigator or clinician is aware of the many factors influencing the EDP, it may be used, in combination with other measurements, in assessing cardiac performance.

(ii) Cardiac Output

Cardiac output can also assist the clinician in evaluating cardiac function. In heart failure the cardiac output may be sub-normal, normal or elevated, but in all cases the myocardial contractility is impaired and the ventricular function curve depressed and shifted to the right.

In acute heart failure the cardiac output falls below normal but in chronic heart failure, of moderate degree, hypervolaemia, mostly due to salt and a water retention, raises the venous pressure (and hence EDV) sufficiently to keep the cardiac output within the normal range. In severe chronic heart failure the cardiac output falls below normal despite further increases in EDV.

Considering the cardiac output in relation to the size of the heart may give a better indication of the heart's condition than considering cardiac output alone. If the cardiac output is normal but the heart dilated (as seen on X-ray) or the EDV grossly elevated, the heart is obviously not functioning normally.

In hyperkinetic states, e.g. anaemia, beri beri etc., heart failure occurs when, despite the high cardiac output, the heart is no longer able to satisfy the tissues demands for blood and myocardial function is impaired by hypoxia. As the heart fails the cardiac output falls from its peak but it is still considerably above normal - hence the term high output failure.

(iii) Cardiac Work

The diagram of ventricular pressure versus volume consists of two curves, which join to form a closed loop representing the cardiac cycle, the area under them represents the integral for ventricular work.

Systolic work, the work done by the ventricle in ejecting blood, is portrayed by the area under the systolic (i.e. upper) curve. Diastolic work, the work done by the blood in filling the ventricle, is represented by the area under the lower curve. The nett work performed by the ventricle is the difference between the systolic and diastolic work (i.e. the area of the pressure volume loop). In ventricular failure, with a high EDV, increased work is expended in distending the ventricle during diastole relative to systolic work, so nett work decreases.

More conveniently, if less accurately, cardiac work is frequently calculated as the product of mean arterial pressure (plus kinetic energy factor) and stroke volume. The kinetic energy of the LV accounts for 0.2-2.0 % of the total useful work of the ventricle, so is frequently omitted, while on the right side it is responsible for 2.4-22.5 % of the useful work of the ventricle and should be included.

Although these formulae are adequate under normal conditions, if a valvular lesion is present the external work of the heart is less than the total work performed by the ventricles. In aortic or pulmonary stenosis a considerable portion of the energy of the ventricle is expended overcoming the resistance of the stenotic area, so the average intraventricular ejection pressure should be used to calculate ventricular work instead of the mean arterial pressure. In valvular insufficiency the total work is equal to the product of the mean ejection pressure in the ventricle and the sum of the stroke volume and regurgitant flow. (Meerson 1969).

(iv) Ventricular Function Curves

In the normal heart as the EDP rises stroke volume or stroke work increase in a curvilinear manner and the ventricular function curve is steepened and shifted upwards, and to the left by inotropic agents. In heart failure the curve is flattened and displaced downwards,

so a substantial increase in the EDV produces a less than normal augmentation of stroke volume.

Ventricular function curves have been determined in experimental animals during infusion of blood or plasma expanders but, as this was only possible if reflex circulatory adjustments were blocked pharmacologically, this method of evaluating cardiac performance is not useful in man. Although EDP is not always a good index of EDV, particularly in chronic heart disease, directional changes can be used to indicate similar changes in EDV and these correlated with changes in stroke volume, can be compared with directional alterations in hemodynamic parameters in other groups of patients. Various tests have been devised in which abnormal responses of the LV can be detected.

Reduced Preload

A partially inflated balloon catheter placed in the inferior vena cava reduces the venous return and the LVEDP - this is accompanied by a great reduction in stroke volume in normal patients but only a small decrease or no change in those with depressed left ventricular function. (Ross 1964 a).

Increased Afterload

A pressor agent with no positive inotropic effect produces an increase in systolic arterial pressure (usually approximately 50 mm Hg) and a substantial increase in LVEDP resulting in an increased stroke volume in normal patients, but a decreased stroke volume in those with LV myocardial disease. This test therefore pushes LVs with myocardial disease onto the descending limb of the ventricular function curve, but this situation is only temporary. (Ross 1964 b).

Exercise

Muscular exercise pushes the ventricular function curve up and to the left - due to the release of catechclamines and stimulation from the sympathetic nervous system. In patients with

no myocardial disease an increase in stroke volume is accompanied by no change or a fall in EDV, in mild myocardial disease an increase in stroke volume is accomplished by increasing the EDP but in severe myocardial disease the increase in EDP is not able to increase the stroke volume. (Ross 1966).

Atrial Pacing

During atrial pacing the cardiac output remains unchanged over a wide range of heart rates and a linear relation exists between LVEDP and stroke volume in each patient at different rates. Patients with abnormal ventricular function are easily detected as they exhibit only small increases in stroke volume for large increases in LVEDP. (Parker et al. 1970).

(b) Cardiac Muscle Performance

Traditionally the heart's performance has been judged by its ability to pump oxygenated blood to the metabolizing tissues, and it was thought to have failed when it could no longer meet these demands. Standard methods of evaluating cardiac performance, based on the characteristics of the ventricles as pumps, were all limited by the associated effects of abnormal loading. It is often not possible to determine whether the inadequate function is due to excess pressure or volume loading, or to an intrinsic abnormality or impairment of the myocardium. The need to find methods of determining the contractility of the myocardium per se led to the consideration of the intact ventricle in terms of muscle function, rather than pump function.

Many indices of contractility have been suggested, while some have proved unsuitable others are still being tested. Examples of some of these are:-

(i) Ejection Fraction

As a chronic pressure or moderate volume load caused no reduction in the ejection fraction of the ventricle until myocardial failure occurred, the ejection fraction was proposed as an index of myocardial contractility. It was subsequently discovered that the ejection fraction was influenced by heart rate, preload and afterload as well as contractility, and that changes in this index and the contractile state could occur independently of each other and in the opposite direction. (Krayenbuhl 1968).

(ii) Peak dP/dt

Peak rate of intraventricular pressure rise (peak dP/dt) generally occurs at the time of opening of the semilunar valves and correlates directly with the contractile state of the ventricle, provided it is computed with recordings made with a high fidelity catheter manometer system and the preload and afterload are carefully controlled. However, alterations in ventricular end-diastolic pressure or arterial diastolic pressure will result in changes in peak dP/dt, so certain hemodynamic variables have been combined with it to try and cancel these changes. In the presence of changes in EDP, not associated with alterations in arterial diastolic pressure, changes in contractility can be determined using the ratio of peak dP/dt to integrated systolic isovolumic tension or to peak isovolumic pressure, maximal isovolumic tension or ventricular end-diastolic pressure. (Mason 1969).

(iii) dP/dt At Common Isovolumic Ventricular Pressure

When dP/dt is determined at the isvolumic ventricular pressure of 50 mm Hg and this ratio is then related to the EDP, accurate assessment of the contractile state can be made. This measurement can be used to study interventions in single patients and to make comparisons between patients. (Mason et al. 1969).

(iv) Contractile Element Velocity. (VCE) at Peak Tension

The velocity of shortening of the circumferential fibres is calculated at peak tension during ventricular ejection by visualization of the ventricular chamber by cineangiography. As Vse (series elastic element velocity) is zero at peak tension, the fibre shortening rate is equal to Vce at this point. Although Vce at peak tension is influenced by variation in muscle lengths it provides quantitative information about myocardial function which can be used when making comparisons between different patients, but not for studying interventions in single patients, as the angiographic dye alters the inotropic state and ventricular preloading is increased for a long period. This technique is difficult to utilise clinically as it requires complicated equipment, complex analysis and accurate knowledge of ventricular tension, which is very difficult to obtain. (Gault et al. 1968).

(v) Maximum Velocity of Contractile Element Shortening (Vmax)

More recently V max (maximum V_{CE}, i.e. V_{CE} at zero load) has been suggested as an index of contractility. This index can be calculated in the conscious patient using only a high fidelity catheter manometer system. V max is derived from the plot relating (dP/dt)/KP (K series elastic modulus = 28 muscle lengths/sec) to the corresponding intraventricular pressure, during the isovolumic phase of the normal ejecting beat, extrapolated to zero load (zero pressure). In the intact heart during isovolumic contractions, alterations in ventricular geometry are very small so knowledge of ventricular radius and wall thickness are not necessary and, as V_{CE} can be assumed to equal V_{SE}, no knowledge of ventricular tension is required. This index has been found to be a sensitive indicator of contractility, when comparing patients, but cannot be used if there is not a truly isovolumic portion of systole (e.g. patients with mitral insufficiency, severe aortic insufficiency or ventricular septal defect). (Mason 1970).

3. METHOD

3. METHOD

(See Appendix at end of Method for list of equipment and drugs)

Right and left ventricular hypertrophy were produced by constriction of the pulmonary artery and aorta respectively. The problem of combating an endemic infection of Chronic Respiratory Disease and choosing and delivering an inhalation anaesthetic proved as difficult as developing a reliable operative technique. To evaluate the performance of these hearts, cardiac output and intraventricular pressures were followed as the hearts were stressed by volume overloading. As no reference could be found describing the recording of intracardiac pressures in the rat via intravascular catheterisation, reliable techniques had to be developed.

A. Production of Right and Left Ventricular Hypertrophy

(1) Chronic Respiratory Disease

The rat suffers from few bacterial or viral diseases and is not subject to many diseases which are highly pathogenic in other species. Despite this remarkable resistance to infection, it is highly susceptible to the variety of etiological agents responsible for Chronic Respiratory Disease (CRD).

CRD was endemic in the white rats supplied to this laboratory, dramatically increasing the post-operative mortality rates to unacceptable levels, elevating the pressures on the right side of the heart and, in severe cases, producing right ventricular hypertrophy (RVH). It was obvious that accurate values for intraventricular pressure and ventricular weights in normal rats could not be established while this disease was present. Production of ventricular hypertrophy in a usable number of rats was also impossible and, in those surviving surgery, it was difficult to determine whether changes in ventricular weight and intraventricular pressure were due to chronic pneumonia or arterial constriction. For these reasons priority was given to attempting to eliminate this disease from the rat colony before serious experiments were commenced.

CRD is a disease of the respiratory system of rats, mice and Guinea pigs; it has an insidious onset, slow protracted course, and is inevitably fatal after a couple of years. There is still considerable controversy over the role of the various pathogens isolated in this disease. Nelson (1967) considers that CRD is a complex of 2 entities, Infectious Catarrh caused by Mycoplasma pulmonis and Enzootic Bronchiectasis (Endemic Pneumonia) due to a virus - these may occur separately or co-exist in the same host. The original infection may be complicated by such secondary invaders as Bordella bronchiseptica, Streptobacillus manitiformis, Bacillus muris, Pasteurella multocida muris Pasteuralla pneumotropica, Diplococcus type 3 and other mycoplasma. (Baer and Preiser 1969; Brennan 1969). It is spread by droplet infection.

Clinical signs of CRD include serious nasal and ocular discharge, red-stained encrustations around eyes and nose, rough hair coat, weight loss, dyspnoea circling or twisting. Unfortunately these signs are not a reliable guide to the presence, or severity (in the early stages) of infection as frequently they do not appear until the animal is about to die.

Pneumonia and rhinitis are the commonest pathological findings, occasionally accompanied by otitis media or otitis externa. In the early stages of the disease, small, discrete grey pink lesions are disseminated throughout the lungs, later these coalesce and whole lobes assume a shrunken grey-pink appearance. Microscopically these lesions consist of bronchiectic cavities containing mucoid and caseous debris accompanied by perivascular and peribronchial lymphoid infiltration; with the arrival of secondary bacterial invaders, infiltration by polymorphnucler leukocytes and abscess formation takes place. (McPherson 1966; Innes 1967).

CRD is endemic in North Eastern America and can only be completely eliminated from a specific colony by the careful breeding and isolation of several generations of rats. As these measures were not feasible for this

experiment, efforts were made to reduce the incidence of the disease in the animals used, to minimize the spread of infection during the operation and finally, all results obtained from rats showing macroscopic evidence of pneumonia at autopsy were discarded.

The rats were obtained from the colony where the incidence of CRD was lowest at the time of the experiments, any animals showing clinical symptoms were destroyed on arrival and the remainder placed in disinfected cages, isolated from the other animals in the building. They were observed for the next ten days, receiving terramycin in the drinking water (see Appendix B10.) for the first five days; any animal which failed to gain weight or showed any other sign of disease was destroyed and its cage removed.

In general, only minimal attention to cleanliness is necessary when operating on parts of the rat's body which are resistant to most pathogenic agents. However, stricter standards are necessary for thoracic surgery, not only to avoid the spread of CRD, but also to prevent the formation of profuse fibrous tissue, which takes place whenever a foreign body is left in the thorax and which, by distortion of the heart and great vessels, may alter the intracardiac pressures and make cardiac catheterisation more difficult.

On the morning of the operation the surgical sites were shaved with an animal clipper equipped with a fine cutting blade (see Appendix, A1) while the rat was restrained by the scruff of the neck; this helped to prevent hair entering the wound. Whilst the endotracheal tube, face mask, autoclips and autoclip applicator were kept under alcohol, the rest of the instruments and the drapes were wrapped in tray clothes and sterilized in an instrument sterilizer (A2) before each operation. An assistant wearing sterile gloves laid the instruments out on trays - placing them on and covering them with sterile clothes. The instruments were divided into two trays - the first was used for intubation (A3), where it was impossible to

preserve sterility but, as CRD normally enters via the upper respiratory tract, it was important to sterilize these instruments between animals. The second tray was used for the sterile thoracotomy (A4), once the rat was attached to the anesthetic machine, the surgeon put on sterile gloves and worked from this tray. The thoracic site was swabbed with Metaphen Tincture (B5) and draped with sterile drapes.

Post-operatively the rats were given 0.2 ml Combiotic (B1) intramuscularly for 3 days.

(2) Anaesthesia

The anaesthetic agent sought for the thoracotomies was one which would enable the animal to be intubated easily, maintained at a surgical level of anaesthesia, without undue depression of respiratory or cardiovascular systems and would allow a rapid recovery. The two anaesthetics commonly used for surgical procedures in the rat, Pentobarbital sodium and ether, were both unsuitable.

When Pentobarbital sodium (B2) was injected intraperitoneally at 40 mg/kg intubation could be performed in 20 minutes but the recovery period was very long, half to one hour. Throughout this period the severe depressive effect of the barbituate on the respiratory and cardiovascular systems, combined with the stress placed on these systems by the thoracotomy and its after effects, resulted in a very high mortality rate.

Ether is a volatile anaesthetic so the level of anaesthesia could be more carefully controlled than that of the barbituate and the anaesthetic stopped at an appropriate time to permit rapid recovery: but two factors decided against its use.

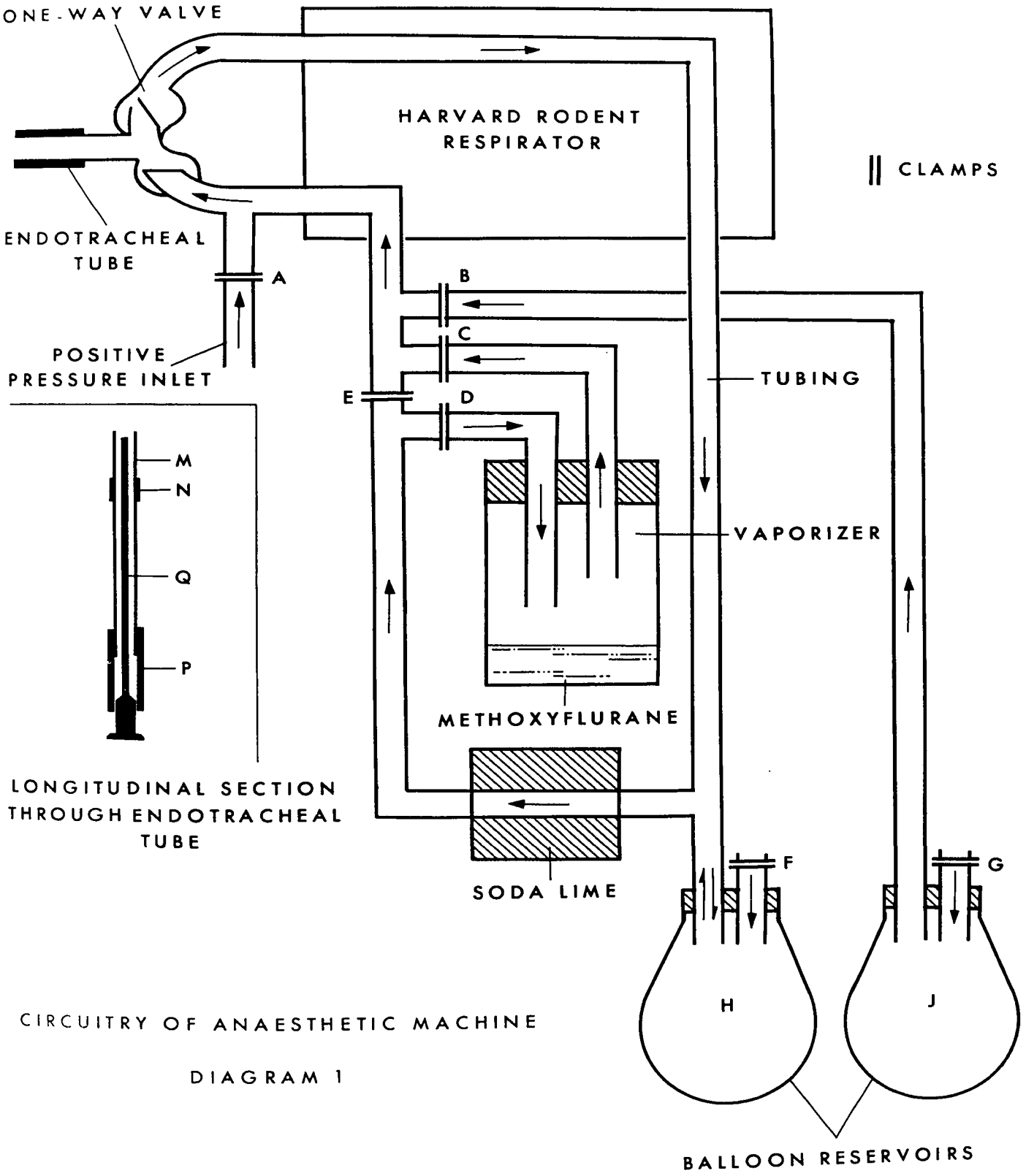
(i) Ether is extremely irritating to the mucous membranes and resulted in an accumulation of watery secretions in the respiratory tract and endotracheal tube. Pre-medication with atropine (B3) an hour prior to operation prevented this side effect but added considerably to the time and effort of the whole procedure.

(ii) Ether is highly inflammable and explosive and it seems ridiculous to use such a dangerous anaesthetic when safer more effective ones are available.

Methoxyflurane (B4), a halogenated ethyl methyl ether, was chosen; this is a volatile anaesthetic with all the concomitant advantages but does not irritate the mucous membranes and is non-inflammable and non-explosive. It has a good reputation in human and small animal anaesthesia and gave excellent results in rats. A smooth induction was possible, with no period of excitement, intubation was satisfactorily accomplished and due to its profound analgesic properties the level of anaesthesia necessary for surgical intervention was much lighter than that required with either ether or Pentobarbital sodium and hence placed much less strain on the respiratory and cardiovascular systems. Recovery was extremely rapid and a significant degree of post-operative analgesia was obtained. (Crawford 1969; Weingarten 1969; Simmons and Smith 1968).

There are reports of administration of positive pressure anaesthesia in the rat via both endotracheal tubes and face masks; the latter were combined with abdominal binders or an oesophageal balloon catheter to prevent gas entering the thorax. (Porter and Small 1947; Loring 1952; Ellis et al. 1952). As the face mask methods were found to reduce the tidal volume considerably the more efficient endotracheal tube was used.

Although insertion of the endotracheal tube via a tracheotomy was quite satisfactory for acute experiments, it was not for the thoracotomies. Post-operatively blood entered the trachea and the neck muscles did not invariably close the wound completely; thus respiration was considerably embarrassed during the vital recovery period. Intubation of the rat under direct vision, as described by Porter and Small (1947) was possible but time consuming and frequently resulted in damage to the larynx and pharynx.



CIRCUITRY OF ANAESTHETIC MACHINE

DIAGRAM 1

A reliable method of intubation was established in which the ventral neck muscles of the anaesthetised animal were separated and retracted to expose the larynx and trachea so the endotracheal tube could be easily guided through the pharynx and larynx and down the trachea. The endotracheal tube consisted of a 50 mm length of polyethylene tubing (A5) (M Diagram 1), with a piece of rubber tubing at one end (p, Diagram 1) to attach it to the respirator and a piece of rubber tubing encircling it 10 mm from the opposite end (n, Diagram 1) to block the larynx. During intubation a stylet (q, Diagram 1) made of a blunted 18 gauge needle, was inserted to stiffen it. Once the tube was safely in the trachea the stylet was withdrawn, the tube sutured to the right commissure of the lips, cleared of debris by suction and attached to the respirator. The retractors were removed from the neck muscles, the skin temporarily closed with forceps and finally sutured after the thoracotomy.

As methoxyflurane is a fairly expensive anaesthetic, a closed circle system was used for both Induction and Maintenance of anaesthesia (see Diagram 1 for circuitry of anaesthetic machine). A Harvard Rodent Respirator (A6) pumped gases from the balloon reservoir (H, Diagram 1), along polyethylene tubing, through a soda lime container, then either through or past the methoxyflurane vaporizer to the one-way valve and to the animal. As only the elastic recoil of the animal's lungs returned the expired gases through the one-way valve and back to the balloon reservoir (H) the resistance on the expiratory line was reduced as much as possible.

The balloon reservoir was filled with 100 % oxygen and the respirator set to deliver $4\frac{1}{2}$ - 5 cc per stroke at 80 strokes per minute in the 450-500 g rats used in the final experiment. During induction all gases passed over the surface of the methoxyflurane in the vaporizer. The rat was induced with a face mask made from a plastic cylindrical container which fitted snugly over the rat's nozzle at one end and was attached to the

one-way valve at the other. The rat was restrained in a crouching position with the mask held firmly over his nose and mouth until the stage of conscious analgesia was reached, then he was tied on his back and the mask held in place until he was deep enough to enable intubation to take place. Once the endotracheal tube was attached to the respirator, clamps C and D were closed and clamp E opened so that all the gases by-passed the vaporizer. Due to the profound analgesic properties of methoxyflurane the level of anaesthesia required for surgical intervention is much lighter than that needed with conventional anaesthetics; so the anaesthetic already in the system, as well as that which was slowly released from the animal's fat stores, was sufficient to maintain anaesthesia during the thoracotomy.

As soon as closure of the thoracic wall was commenced, clamp E was closed and B opened so the animal was breathing pure oxygen from the balloon reservoir (J, Diagram 1) during the sewing-up. By the time the skin clips had been applied the rat was at the stage of conscious analgesia and movement, when disconnected from the respirator it would breathe spontaneously in 3-4 seconds and regain the righting and swallowing reflex in another 20-30 seconds. The endotracheal tube was then sucked out and removed and the animal placed in an observation cage for 20 minutes before being returned to its normal cage.

(3) Operations for Constriction of Pulmonary Artery and Ascending Aorta

Constriction of the pulmonary artery was chosen to produce RVH as the stimulus responsible for ventricular hypertrophy is isolated, effecting only the right side of the heart and not the other tissues of the body. Although constriction of the ascending aorta is a much more difficult operation than constriction of the abdominal aorta, it was chosen as it only effects the left side of the heart, whereas constriction of the abdominal aorta also reduces the blood supply to the lower portion of the body and the kidney in particular.

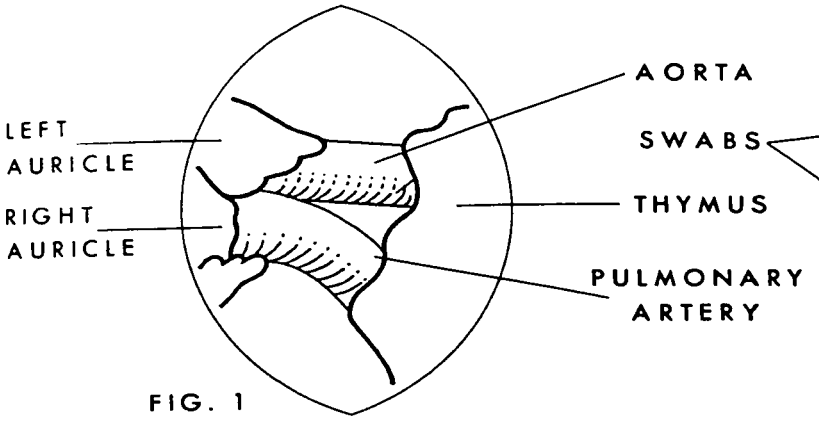


FIG. 1

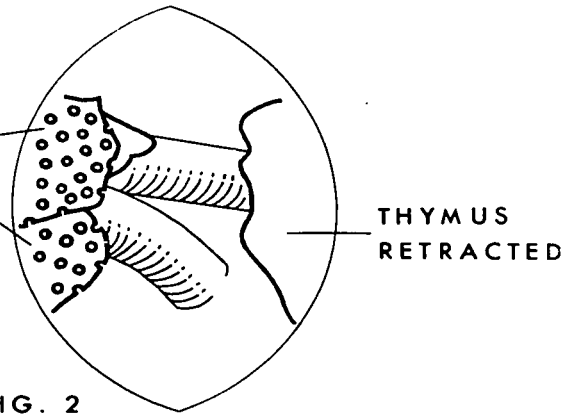


FIG. 2

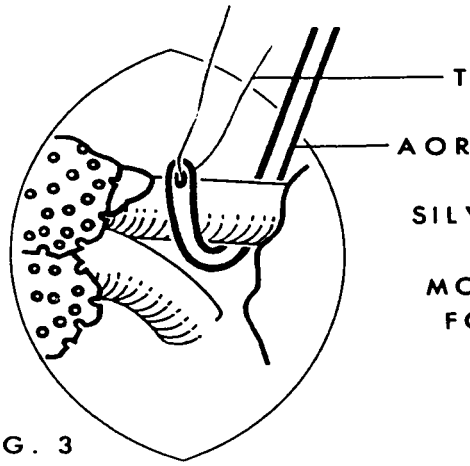


FIG. 3

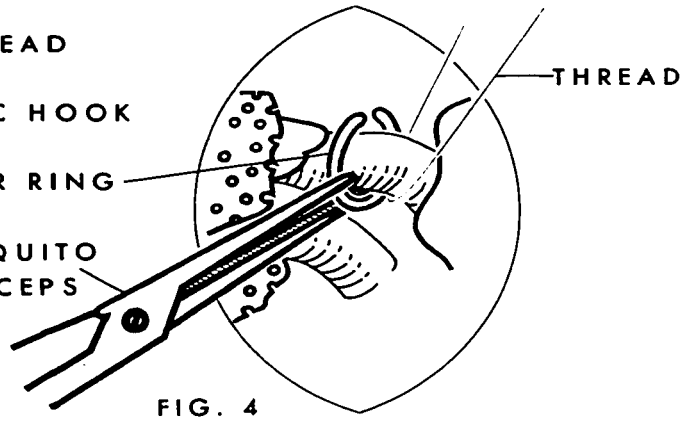


FIG. 4

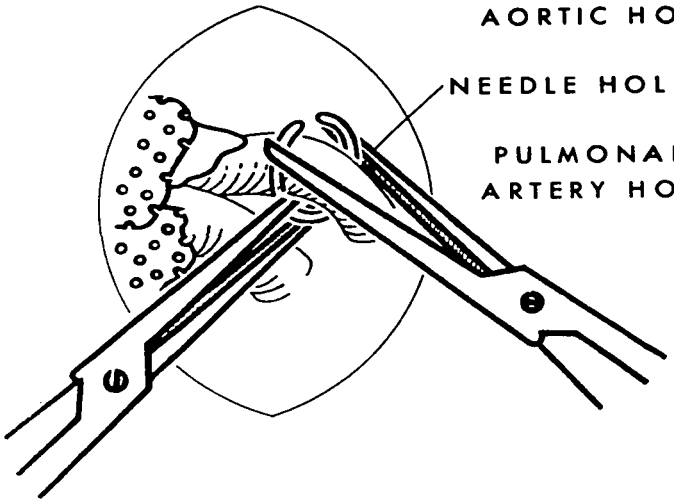


FIG. 5

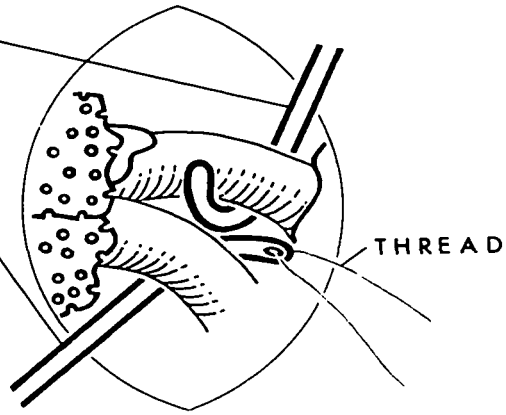


FIG. 6

CONSTRICTION OF AORTA AND PULMONARY ARTERY
(SEE TEXT FOR EXPLANATION)

DIAGRAM 2

A mid-sternal incision was tried initially but this involved considerable trauma which caused profuse intrathoracic fibrosis and was difficult to close. A left lateral incision through the third intercostal space was finally selected, as this afforded good access to both the pulmonary artery and ascending aorta, with minimal trauma and could easily be closed.

Once intubation was completed, the anaesthetic adjusted for maintenance and the neck incision closed with forceps, the animal is tied on its right side with the left foreleg extended anteriorly, bringing the left ventro-lateral surface upwards and the pulmonary artery closer to the thoracic wall. A padded bandage was then tied around the abdomen so the abdominal contents pressed up against the diaphragm.

This abdominal bandage helped to prevent fatal pneumothorax, which had been a frequent complication of early trial experiments. It prevented air, which entered via the thoracic incision, from accumulating between the lungs and the diaphragm throughout the operation. During closure of the chest, pressure on this bandage, combined with inflation of the lungs, helped to expel any unwanted air from the thoracic cavity.

The incision site was swabbed with Metaphen Tincture (B5) and draped before the skin incision was made over the third intercostal space, extending laterally for 3-4 cm. from the edge of the sternum and running parallel to the ribs. The superficial and deep portions of the pectoralis major were then incised to expose the intercostal muscles of the third intercostal space, these were separated by blunt dissection until the lungs could be seen clearly through the transparent pleura. The pleura was carefully pieced, avoiding the left lung and a swab was pushed through to protect it as the incision was enlarged. The final intercostal incision commenced just lateral to the internal mammary artery (which runs along the edge of the sternum) and extended laterally for approximately 3 mm.

The left lung was then packed back with more swabs and the retractors placed in the incision and opened to expose an area of the thymus - approximately 1 x 1.2 cm. The thymus was then reflected cranially exposing the atria and great vessels (see Diagram 2, fig. 1 and 2). If the exposure was not good enough, as was often the case in larger rats (over 400 g) with less flexible ribs, the retractors were closed and the 4th rib cut, approximately 3 mm from the edge of the sternum, and the retractors reopened - this usually gave excellent exposure in larger rats.

The right and left atria were protected with swabs and the ascending aorta raised with the curved end of the aortic hook (a small threaded aneurysm needle) (Diagram 2, fig. 3). A small section of aorta was cleared and held by the thread, as the hook was withdrawn. The aorta was then raised and constricted by the thread, the open end of the silver ring (held by mosquito forceps) was slipped over the narrowed, cleared portion of the aorta. (Diagram 2, fig. 4). The thread was then dropped and the ring closed with long nosed needle holders (Diagram 2, fig. 5) and then released as quickly as possible. After checking that the two ends of the ring were in perfect apposition and that there was no extra tissue under the ring, the thread and swabs were removed and closure of the thorax commenced. The aortic ring was placed on the ascending aorta as close as possible to the aortic valve.

The operation for constriction of the pulmonary artery was the same as that for constriction of the aorta until the aorta was isolated. When the pulmonary artery was to be constricted, the aorta was raised by an unthreaded aortic hook and the tissue connecting it and the pulmonary artery cleared away. A pulmonary hook (a fine threaded aneurysm needle whose end was bent at approximately 45°) was slipped under the pulmonary artery as the aorta was raised and twisted slightly by the aortic hook (Diagram 2, fig. 6) - the thread grasped and both hooks swiftly withdrawn.

A section of the pulmonary artery was cleared, the ring slipped on and closed in the same manner as the aortic ring. The pulmonary artery ring was placed on the main pulmonary artery before it divides into right and left branches.

A check was always made to see that all swabs and threads were removed. These swabs were made from small pieces of cotton wool, fastened to long lengths of thread and were sterilized and moistened before use. If not fastened to thread they worked their way down to the diaphragm, where they were difficult to remove and were easily forgotten.

The left lung was fully re-inflated, by gently blowing down the positive pressure inlet on the inspiratory line (Diagram 1), and then covered with a 2 cm square of soft plastic sheeting, to protect it from needle pricks during the laying of the sutures. A sterile suture of OO silk with a $\frac{1}{2}$ circle atraumatic needle was used to close the chest wall. Four single interrupted sutures were laid to ensure complete closure of the wound. The needle passed through the pectoral muscles, then the muscles and pleura of the second intercostal space, then out the pleura and muscles of the fifth intercostal space and finally out through the pectoral muscles. When all the sutures were laid the plastic cover was removed and the two sutures at either end of the wound were tightened. During the tightening of the last two sutures the lungs were expanded via the positive pressure inlet and the abdomen depressed to expel as much air as possible from the thoracic cavity. The skin incisions were then closed with autoclips.

The operation for constriction of either the aorta or the pulmonary artery took from 15 to 20 minutes. Approximate times for the various stages were Induction 4 minutes, Intubation 2 minutes, Thoracic Operation 7 minutes and Sewing Up 4 minutes.

The rings were made of sterling silver wire (0.8 mm diameter) as previously described by Beznak (1958). The wire was wound around a hypodermic needle in a tight spiral and cut into rings with a jewellers saw, these were then straightened and polished until smooth with fine emery paper.

In the final experiment in which male Sprague-Dawley rats weighing 425-525 g were used, three different ring sizes were tested on each artery. Rings 18, 17 and 16 (made by winding the wire around hypodermic needles of gauges 18, 17 and 16 respectively) of internal diameter 1.13 mm, 1.40 mm and 1.55 mm respectively were tried on the aorta. Rings 16, 15 and 13, of internal diameter 1.55 mm, 1.73 mm and 2.31 mm respectively, were tested on the pulmonary artery. The effectiveness of these rings was tested by weighing the ventricles at the end of a set period of time; these weights expressed per Kg of body weight, were then compared to those obtained from normal rats in the same weight range. The ventricles were then dried in an oven for several days and reweighed to obtain their dry weights.

B. Cardiac Output

Cardiac output was determined by the direct Fick Method, with the animal under Pentobarbital sodium anaesthesia and breathing 100 % oxygen. The oxygen consumption was measured by a microspirometer, the arterial samples were taken from the cannulated femoral artery and then mixed with venous samples from the right ventricle, via a catheter passed down the right external jugular vein. The basal cardiac output was first determined and then further post-infusion measurements were made at set times, as the heart was stressed an intravenous infusion of a PVP solution. (B8)

The rat was anaesthetised with Pentobarbital sodium (40 mg/Kg IP) (B2), tied on its back and the vessels to be cannulated were isolated, cleared and surrounded by loose double ligatures. Next a tracheotomy was performed and a tracheal cannula tied securely in place. Hepartin (B6) (15 mg/Kg) was injected intravenously, through the left external jugular vein to prevent blood coagulation. The right femoral artery was cannulated, about 1 cm after emerging from the abdominal cavity, with the blunted end of an 18 gauge needle, which was connected to a pressure transducer by a 2 way stopcock. The left femoral vein was cannulated, a cm or so before entering the abdomen, with a length of polyethylene tubing (PE 20) (A7), and connected to a syringe containing the PVP solution.

After insertion of needle electrodes, lead 11 of the electrocardiogram was recorded by an electrocardiograph (A8) and monitored on a cathode ray oscilloscope to assist in positioning the cardiac catheters. Catheters were then passed down the right external jugular vein and the right carotid artery to the right and left ventricles respectively - using methods which will be described later. These catheters were then connected to pressure transducers by 2 way stopcocks.

The small plexiglass spirometer was connected to the tracheal cannula by a one-way valve and then filled with pure oxygen; the expired CO_2 and H_2O were absorbed by the Baralyme (B7) and silica gel respectively. The fall in the level of the spirometer bell, as the rat used up the oxygen inside it, was recorded electrically by means of a carrier amplifier of a Grass polygraph. Before each experiment the spirometer was calibrated by withdrawing known amounts of oxygen and measuring the distance moved by the polygraph recording pen.

The blood samples were withdrawn through the side-arms of the two-way stopcocks, the first 150 lambda of blood which had accumulated in the stopcock and catheter was discarded and the next 200 lambda collected. The oxygen saturation was determined immediately using an American Optical Micro-Oximeter (A9). A portion of this sample was then mixed with 3 ml of Drabkins Solution (B9) and the optical density measured by a spectrometer (A10) and the capacity determined. Multiplication of the oxygen saturation by the capacity yielded the oxygen content and hence the arterio-venous difference was calculated.

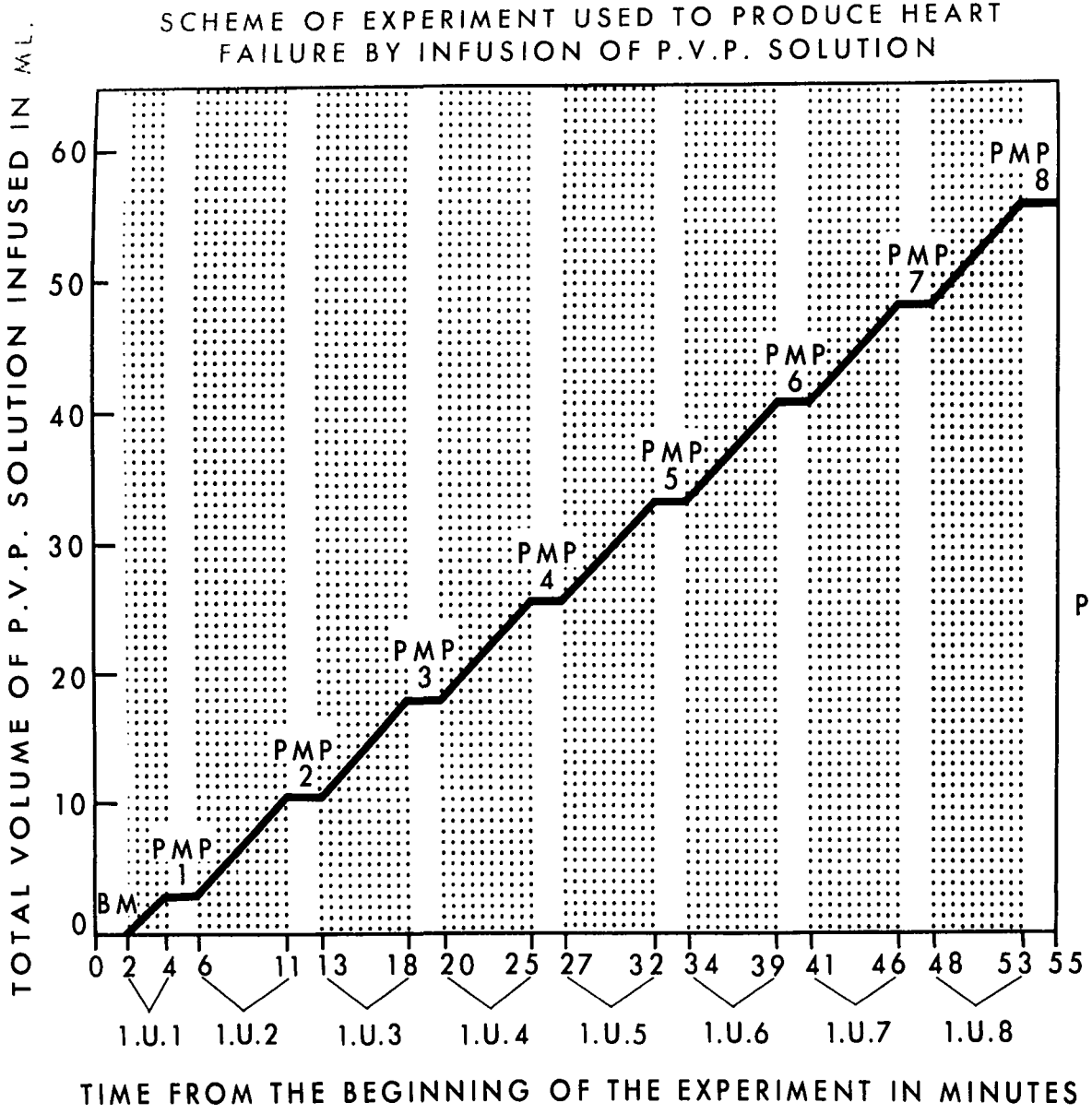
The Micro-Oximeter was very simple to use, before each experiment the zero point had to be checked but no further adjustment was necessary throughout the experiment. The unhaemolysed blood sample was put in a curvette, provided with a stirrer and placed in the light beam. The Micro-Oximeter simultaneously measured the ratio of the light intensities

at two different wavelengths (805 and 650 millimicrons) diffusely reflected from the blood in the curvette, enabling its oxygen saturation to be read directly. The use of two wavelengths rendered the result independent of haematocrit over a wide range of values. (Cole and Hawkins 1967).

Although the Oximeter did not need to be calibrated before each experiment it was originally calibrated by the manufacturers for use with human blood and each time a new lamp was used (approximately every 6 months) it had to be recalibrated for use with rats' blood. Samples of venous rat blood were oxygenated to varying degrees in a bubble tonometer (Ravin and Briscoe 1964) by passing a humidified mixture of 95 % oxygen and 5 % CO_2 through them for different periods of time. The oxygen saturation of each sample was determined by both the oximeter and the spectrometer in combination with the Roughton and Scholander method (1943). The two sets of results were related by a regression equation which was used to determine the appropriate saturation values for the rats' blood which should be substituted for the human values appearing on the oximeter scale.

Basal cardiac output was determined after the rat had been breathing 100 % oxygen for at least four minutes and the respiration was regular. The oxygen consumption was measured for 30 seconds and the blood samples withdrawn during the next 30 seconds, then the infusion pump was turned on.

An infusion, a solution of 12.5 % polyvinylpyrrolidone rendered isotonic with plasma by the addition of electrolytes (B8), was delivered by a Sage infusion pump (A11), via the left femoral vein, at a rate of 1.5 ml/minute. The first infusion period lasted 2 minutes, consisted of 3 ml of solution and was known as the first Infusion Unit (IU 1). The pump was then stopped for 2 minutes to take post infusion measurements, the animal was allowed to settle for the first 30 seconds, the oxygen consumption measured for the next 30 seconds and the blood sample withdrawn during the last 30 seconds.



B.M. BASAL MEASUREMENTS
P.M.P. POST INFUSION MEASURING PERIOD:
I.U. INFUSION UNIT
N.B. INFUSION RATE 1.5ml/min

Figure 1

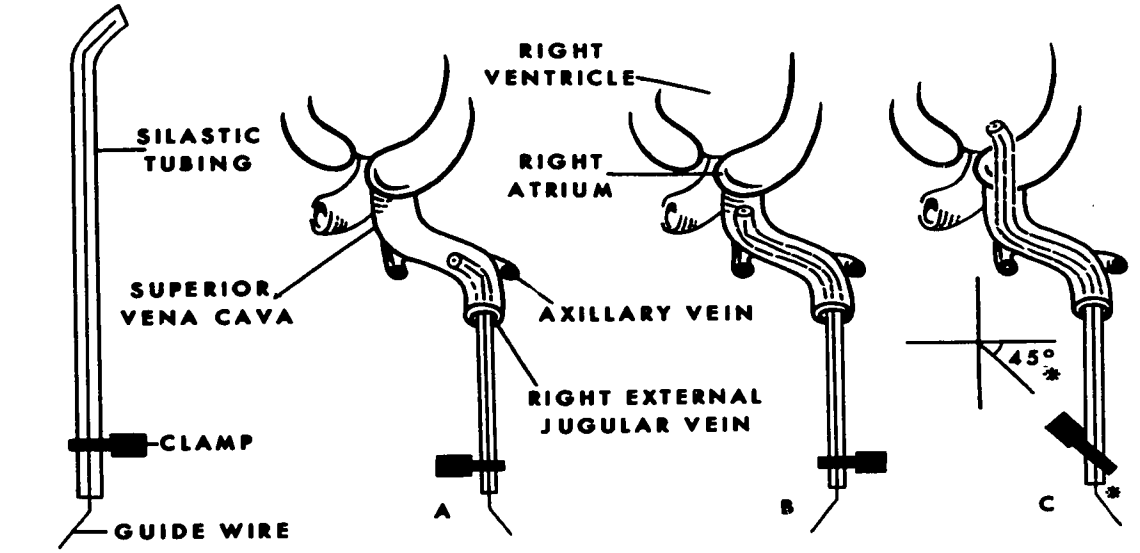
All subsequent infusion units lasted for five minutes, consisted of 7.5 ml of solution and were separated by post infusion measuring periods (PMP), throughout which the pump was stopped for two minutes. The infusion was continued for 8 infusion units (I Us) or until the rat died, whichever was sooner. This experiment was designed by Beznak (1958) for further details see plan on Figure 1.

C. Other Parameters

When the performances of normal and hypertrophied hearts were compared during volume overloading, the changes in femoral artery pressure and intraventricular pressures were recorded as well as those in cardiac output. Although accurate measurements of intracardiac pressures have been obtained by intravascular catheterisation in many species, only inaccurate determinations have been made in the rat by needle puncture of the ventricular wall (Radzievskii and Kapel'ko 1969; Lipana and Fanburg 1970); so new techniques for catheterising the right and left ventricle had to be developed.

Popovic et al. (1963) developed a method for cannulation of the right ventricle in which narrow bore polyethylene tubing (internal diameter 0.011") was advanced down the jugular vein and manipulated into the right ventricle with the aid of a removable guide wire. Depoca (1969) modified this method by using a very light flexible silastic tubing (internal diameter 0.012") which floated in the right ventricle with the returning venous blood. The internal diameter of the tubing used in these two methods was quite adequate for withdrawing blood samples but much too narrow to record an accurate undamped pressure pulse.

An undamped intraventricular pressure pulse was recorded through polyethylene tubing of internal diameter 0.030" - this was a firm tubing and could be manipulated into the right ventricle without a guide wire but the sharp stiff end frequently penetrated vessel and atrial walls or damaged the tricuspid valves. Next a silastic tubing of the same internal diameter was tried, this was a flexible slippery tubing, that required a guide wire


SILASTIC TUBING
CLAMP
GUIDE WIRE
RIGHT VENTRICULAR CATHETER
RIGHT VENTRICLE
RIGHT ATRIUM
SUPERIOR VENA CAVA
AXILLARY VEIN
RIGHT EXTERNAL JUGULAR VEIN
45°
A
B
C
CATHETERISATION OF RIGHT VENTRICLE (SEE TEXT FOR EXPLANATION)

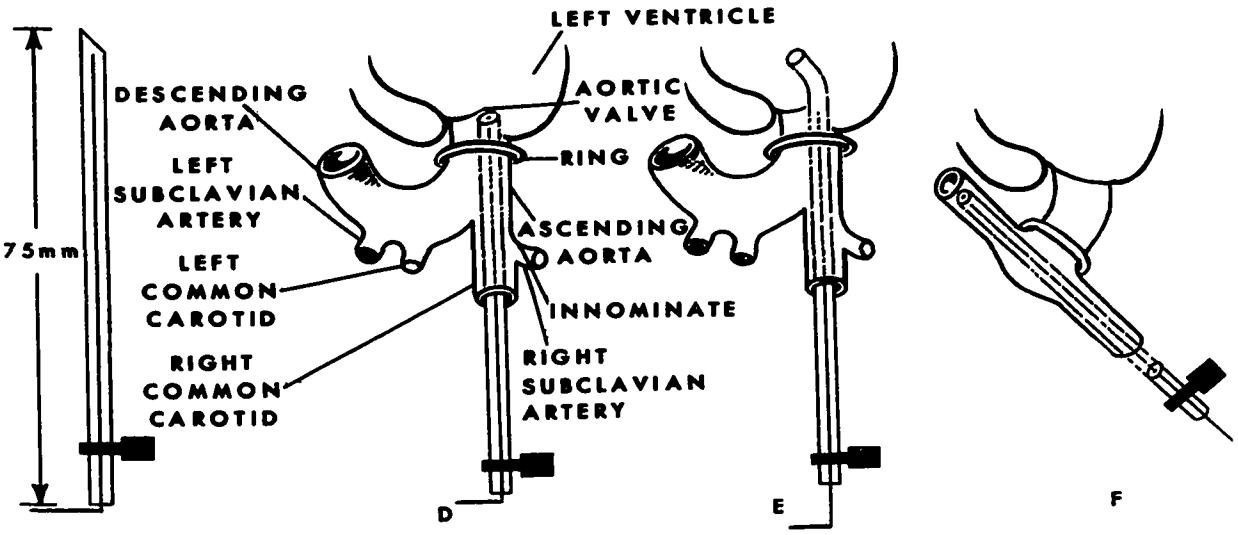

75mm
DESCENDING AORTA
LEFT SUBCLAVIAN ARTERY
LEFT COMMON CAROTID
RIGHT COMMON CAROTID
LEFT VENTRICLE
AORTIC VALVE
RING
ASCENDING AORTA
INNOMINATE
RIGHT SUBCLAVIAN ARTERY
D
E
F
LEFT VENTRICULAR CATHETER
CATHETERISATION OF LEFT VENTRICLE (SEE TEXT FOR EXPLANATION)

DIAGRAM 3

but the end was so soft that it rarely caused any damage. The catheter used in the experiment consisted of a 75 mm length of silastic tubing (internal diameter 0.030") (A12) trimmed to a point at one end with a removable guide wire of fine stainless steel wire, the last 3-4 mm of which was bent at an angle of approximately 66 (A13).

The guide wire was pulled through the tubing until the sharp tip of its bent end lay about 9 mm behind the soft plastic point. The protruding end of the guide wire was bent in the opposite direction, but parallel to its bent end. Silastic tubing is very difficult to mark permanently so the angle of the protruding guide wire was used to indicate the direction of the head of the catheter. The catheter was filled with saline and closed with a clamp (approximately 40 mm from the pointed head). This clamp prevented movement of the guide wire inside the tubing and suction of air into the veins (see Diagram 3).

The animal was prepared as described in section B of the Method. After heparinization and cannulation of the femoral artery and vein, the cranial of the two ligatures surrounding the right external jugular vein was tightened. The pointed tip of the catheter was inserted in an incision made in the jugular vein approximately 5 mm anterior of the thoracic muscles and the caudal ligature fastened loosely around it, to prevent seepage of blood and entry of air. The catheter was advanced with the point directed towards the left - to avoid slipping down the axillary vein (and thus out along the lateral thoracic wall) and to facilitate passage between the first rib and the clavicle (Diagram 3, A). When the catheter tip entered the thoracic cavity, twisting it to the right directed it into the superior vena cava (Diagram 3, B).

As the catheter advanced smoothly down the superior vena cava it was rotated so the tip pointed ventro-medially at an angle of 45 - i.e. the protruding end of the guide wire was directed dorso-laterally. The catheter was held at this angle and advanced gently until it encountered resistance and changes in the ECG monitor indicated that it was touching the ventricular wall. (Diagram 3, C). It was pulled back a few mm and the clamp carefully

released, if blood spurted out the catheter was still in the right ventricle so the guide wire was withdrawn and the caudal ligature tied securely. The catheter's position was finally verified by slipping the free end over an 18 gauge needle, connected to a pressure transducer, and checking that an intraventricular pressure tracing appeared on the oscilloscope screen.

If the saline pulsated at the end of the tubing when the clamp was released and the guide wire was still in place, the catheter tip was only in the atria but if the saline rushed down the tubing, the tip was in either vena cava and the clamp had to be replaced quickly to avoid an air embolism. Further manipulation of the catheter at this stage could place it in the ventricle but an atrial or vena caval trace on the oscilloscope screen indicated that the silastic tubing had to be completely withdrawn, the guide wire re-inserted and the catheterisation procedure started again.

Several workers have cannulated the carotid artery or aorta of the rat (e.g. Popovic and Popovic 1960) but no report has been found of catheterisation of the left ventricle via the arteries. Silastic, rather than polyethylene tubing was used to catheterise the left ventricle as it was flexible and could easily be flipped through the aortic valve, whereas polyethylene tubing damaged the valve every time it was used. The left ventricular catheter consisted of 75 mm of silastic tubing (internal diameter 0.020") (A14) trimmed to a point at one end, with a stainless steel guide wire inserted to within 2 mm of the pointed end. The protruding end of the guide wire was bent at 90° to the tubing to prevent its sharp tip from advancing in front of the silastic covering. A clamp was used to advance the catheter and prevent blood loss; preventing blood loss was a major problem throughout this catheterisation.

The right carotid artery was cleared approximately 1 cm anterior to the thoracic inlet and 2 ligatures placed around it. The cranial ligature was tied tightly and a secure clamp placed beyond the caudal one.

An incision was made between the ligatures, the catheter inserted and the cranial ligature tightened around it to prevent blood loss but allow movement. The caudal clamp was removed and the catheter advanced rapidly until 35 mm of its length had entered (ie. 40 mm still protruded) and its tip was just above the aortic valve. The guide wire was pulled back, leaving a flexible tip of 5-10 mm, this tip was gently manipulated until it slipped through the aortic valve and the catheter slid easily into the ventricle. Its entry was heralded by ventricular premature contractions on the ECG monitor, which settled down in a minute or so. The catheter was securely tied, the guide wire removed and the end slipped over a 21 gauge needle and attached to a pressure transducer. If an aortic, instead of an intraventricular, pressure tracing was recorded the catheter could be partially withdrawn, the guide wire re-inserted and another attempt made.

In this experiment the catheter had to pass through the aortic ring when catheterising animals with LVH. If the ring was placed too close to the right carotid artery the catheter could not negotiate the firm bend thus formed and, instead, passed down the descending aorta (Diagram 3, F). In normal animals, and those whose aortic rings were placed close to the aortic valve, this complication never occurred. In these animals the catheter had no trouble passing through the size 17 and 16 rings which were used in the experiment.

The femoral artery and, left and right ventricular pressures were all recorded by means of Statham P23 Dd transducers and the Electronics for Medicine Medical Recorder (A15). The frequency response of the right and left catheters, attached to their transducers by connecting needles was measured using the pressure generator described by Shelton and Watson (1968).

Each Electronics for Medicine amplifier was equipped with calibration signals, which were recorded prior to the pressures. Initially these calibration signals were checked with a mercury manometer and if not perfectly accurate an appropriate calibration factor was calculated.

These calibration factors were checked every couple of months and did not vary significantly. Before each experiment the positions of zero pressure for right and left ventricular pressures were adjusted until approximately level with the mid-ventricle on each side of the heart. The recordings were made photographically and had to be developed after each experiment.

All pressure recordings - both basal and post-infusion measurements - were made in the same thirty seconds that oxygen consumption was measured for the cardiac output determination. For the first fifteen seconds right and left systolic pressures were recorded and for the next fifteen seconds the amplitude was increased to more accurately record the right and left end-diastolic pressure. The mean femoral artery pressure was recorded throughout this period, except for approximately 10 seconds when the pulse pressure was recorded.

Between post infusion measuring periods the end-diastolic pressures were monitored continuously at a very slow speed.

After the main experiment a number of catheterisations were performed in which the intra-ventricular pressure tracings were examined in detail and particular attention was paid to the pressure changes taking place during LV pullouts.

As well as the parameters already mentioned, two others were considered which were derived from these basic values. Cardiac work was calculated as the product of the cardiac output and the mean arterial pressure. Only the work of the left ventricle was considered, although only the output of the right ventricle was measured and the mean femoral artery pressure was taken as the mean arterial pressure. (NB. the kinetic energy factor was omitted). In animals with aortic rings the mean arterial pressure was determined by taking the mean value of the LVSP and the diastolic femoral artery pressure.

D. Preliminary Trials

(1) Electrocardiography

The electrocardiography of the rat has been studied under normal and pathological conditions by a number of different workers. Although there are descriptions of the electrocardiogram seen in rats with LVH (Beznak et al. 1944; Uhley 1961) none could be found of those occurring with RVH. Accordingly preliminary trials were undertaken to try and determine if rats with cardiomegaly, produced by the methods just described, could be used to study the electrocardiographic changes occurring during the development of right and left ventricular hypertrophy.

The electrocardiogram of all rats was recorded 15 to 20 minutes after the injection of Pentobarbital sodium (40 mg/Kg), just prior to the cut down procedure for insertion of cannulae. Initially ECGs were recorded with the rats in the supine position - in both positions the legs were tied in full extension. Needle electrodes were inserted at the distal end of the fore and hind legs and the ECG was recorded on a Hewlett Packard Electrocardiogram (A8) (which had a frequency response of 3db down at 100 cps) at a paper speed of 50 mm/sec and a calibration of 0.5 mv/cm. In some cases recordings were also made on the Electronics for Medicine Medical Recorder (A15) at a paper speed of 80 mm/sec and a calibration of 0.25 mv/cm. In all rats the standard limb leads (leads 1,11 and 111) and augmented limb leads (aVR, aVL and aVF) were recorded and the QRS axis determined.

(2) Contractility

Preliminary trials were undertaken to try and determine if it would be possible to use normal rats and those with experimental ventricular hypertrophy to determine simultaneously the contractile state of both ventricles, using only catheter transducer systems. Contractility of the normal, hypertrophied and failing LV has been measured, in other species, but little work has been done on the normal or hypertrophied RV and studies comparing the simultaneous contractile state of both ventricles are lacking.

In these trials force-velocity curves were created by phase-plane plotting the intravenous pressure (P) versus the fraction of the first pressure derivative (dP/dt) divided by the corresponding pressure (P) during the isovolumic phase of a normal ejecting or auxotonic beat. Extrapolation of the descending limb of the curve to zero load (zero pressure) allowed estimation of the maximal intrinsic velocity of the contractile element (V max).

As this was only intended as a preliminary trial the number of animals in each group was not large enough to be analysed statistically for significance.

APPENDIX TO METHOD

A. Equipment

1. "Oster Model A2 Animal Clipper" with cutting blade size 40
   (John Oster Manufacturing Co., Milwaukee, Wisconsin, U.S.A.)

2. Instrument Sterilizer (Sichheitsausschallung, Germany)

3. Tray 1 - for Intubation.
   (Sterilized in Instrument Sterilizer)
   (1) 2 x Tray clothes white linen, 20 x 35 cm.
   (2) Tissue Forceps 5".
   (3) Scalpel handle no. 3.
   (4) Extra fine microdissecting forceps, 4", curved.
   (5) 2 x Allis tissue forceps - 6".
   (6) Kelly haemostatic forceps - 5".
   (7) Fine pointed dissecting scissors, curved - $4\frac{1}{2}$".
   (8) Cotton wool swabs.
   (Added later)
   (9) Scalpel blade no. 11.
   (10) Suture needle, half circle cutting.
   (11) Endotracheal tube.

4. Tray 2 - for thoracotomy.
   (Sterilized in Instrument Sterilizer)
   (1) 2 x Tray clothes white linen - 20 x 35 cm.
   (2) Drape 40 x 25 cm - central cut out area 4.5 x 2.5 cm.
   (3) Backhaus towel forceps - $3\frac{1}{2}$".
   (4) Scalpel handle no. 3
   (5) Adson forceps - $\frac{1}{4}$".
   (6) Curved blunt forceps $4\frac{1}{2}$".

A. Equipment (Continued)

4. (7) Extrafine microdissecting forceps, curved, 4".

(8) Fine pointed dissecting scissors, curved, 4".

(9) Heiss retractors, 4", 4 x 2 prongs (rubber tipped)

(10) Halstead mosquito forceps with silver ring.

(11) Aortic hook.

(12) Pulmonary artery hook.

(13) Needle holder long nosed.

(14) Needle holder.

(15) 4 x serrefines, $2\frac{1}{4}$".

(16) Cotton wool swabs.

(Added later)

(17) Scalpel blade no. 11.

(18) Autoclip Applier and Autoclip wound clips - 9 mm.

(19) Davis and Geck sterile suture - $\frac{1}{2}$ circle taper atraumatic needle, 30" non-capillary silk 00.

(20) Small plastic sheet, 2 cm square.

5. Polyethylene tubing 240, internal diameter 1.67 mm external diameter 2.42 mm.

6. Harvard Rodent Respirator, Harvard, U.S.A.

7. Polyethylene tubing P.E. 20, internal diameter 0.38 mm external diameter 1.09 mm.

8. Hewlett Packard Electrocardiograph, IS - 1500 A-1 (Hewlett Packard, U.S.A.)

9. 10850 Micro-Oximeter (American Optical Company, Medical Division, Chelsea, Mass., U.S.A.)

A. Equipment (Continued)

10. Spectronic 20 (Bausch and Lamb)

11. Variable Infusion Pump (Sage Instruments, White Plains, U.S.A.) model 241, with 50 ml syringe, plunger siliconised and oiled.

12. Silastic medical grade tubing (Dow Corning Corp., Michigan, U.S.A.) internal diameter 0.030", external diameter 0.065".

13. Stainless steel stylets for blood pipettes (Clay Adams)

14. Silastic medical grade tubing (Dow Corning, Michigan, U.S.A.) internal diameter 0.020" and 0.037".

15. Electronics for Medicine 5 z Channel Medical Recorder -PR 8 (Electronics for Medicine, White Plains, N.Y., U.S.A.)

B. Drugs

1. "Combiotic" (Pfizer, each ml contains 200,000 Units procaine penicillin G and 0.25 g dihydrostreptomycin sulphate).

2. "Nembutal Powder for Injection". (Abbott Laboratories Ltd., Montreal) Pentobarbital sodium powder, Dose 40 mg/Kg I. P.

3. Atropine sulphate, Dose 2.0 mg per rat.

4. "Penthrane". (Abbott Laboratories, Montreal) Methoxyflurane (2,2 Dichloro 1,1 Difluoroethyl methyl ether).

5. "Metaphen Tincture" (Abbott Laboratories, Montreal) Nitomersol 500 mg in 100 ml of alkalinized solvent.

6. Heparin sodium (Fisher Scientific) 1 g = 160,000 IU, Dose rate 15 mg/kg I P.

B. Drugs (continued)

7. "Baralyme Granules" (McGraw Edison Co) Barium hydroxide lime USP.

8. 37.5 % Polyvinylpyrrolidone (PVP) (Poulenc Ltd., Montreal)

To make 100 ml of infusion -

0.9 % NaCl	50.0 ml
10 % NaCl	1.27 ml
5 % $NaHCO_3$	4.20 ml
5 % KH_2PO_4	1.40 ml
5 % $MgSO_4 \cdot 7\ H_2O$	0.59 ml
37.5 % PVP	30.0 ml
5 % $CaCl_2$	0.56 ml
Add until 800 ml 0.9 % NaCl	

9. Drabkins Solution

Na HCO_3	1.00 g
KCN	0.05 g
$K_3Fe(CN)_6$	0.20 g

Distilled water to 1000 ml.

4. RESULTS

4. RESULTS

A. Production of Cardiomegaly

(1) Normal Rats

The rats used in this experiment were all male white laboratory rats, of the Sprague Dawley strain, ranging in weight from 428 to 525 g; the mean weight of the control group was 473 ± 7g and the mean weight of the other groups did not differ significantly from this value. As there was no significant difference between the heart weight, body weight ratios of the 6 sham operated rats and the 18 normal rats these results were pooled to form a control group. The right ventricular weight of the control group was 0.0660 ± 0.0020 gRV/100 g BW, the left ventricular weight was 0.1577 ± 0.0031 gLV/100gBW, and the ratio of right to left ventricle was 0.421 ± 0.011.

The circumference of both the aorta and pulmonary artery, measured in 6 rats, averaged 8 mm (diameter 2.6 mm); these results were very difficult to obtain and could only be regarded as rough estimates of the actual values.

The body weight of normal rats in this weight range increased by approximately 35 g in a 10 day period, 42 g in 12 days and 50 g in 14 days.

(2) Sham

There were no deaths during or after the sham operation. In the two weeks following the operation these rats gained an average of 35 g. At the time of catheterisation the heart weight, body weight ratio (HW/BW) did not differ significantly from that of the normal group.

Changes in Weight of Right and Left Ventricle with Different Rings - Table 2

Group	Number of rats	Body Weight	gRVW/100g BW	gLVW/100g BW	RVWg/LVWg
CONTROL	24	473 ± 7	0.0660 ± 0.0020	0.1577 ± 0.0031	0.421 ± 0.011
AORTIC RING 16	11	NS 495 ± 3	NS 0.0649 ± 0.0011	*** 0.2035 ± 0.0045	*** 0.333 ± 0.013
AORTIC RING 17	5	NS 471 ± 5	NS 0.0712 ± 0.0047	*** 0.2161 ± 0.0086	** 0.332 ± 0.027
P.A. RING 15	10	NS 462 ± 7	*** 0.0997 ± 0.0045	NS 0.1472 ± 0.0037	*** 0.681 ± 0.033
P.A. RING 16	5	NS 445 ± 14	** 0.0917 ± 0.0077	* 0.1324 ± 0.0035	*** 0.695 ± 0.062
P.A. RINGS 15 + 16	15	NS 456 ± 7	*** 0.0971 ± 0.0039	NS 0.1423 ± 0.0032	*** 0.686 ± 0.030

NB Control Group consisted of 18 normal rats and 6 sham operated rats. The heart weights of these two groups were not significantly different.

P.A. RING (pulmonary artery ring). Although the heart weights of rats in group P.A. 15 and P.A. 16 were significantly different their cardiac outputs and intraventricular pressure values did not differ significantly so they were treated as one group in later analysis.

g RVW (right ventricular weight in gram) g LVW (left ventricular weight in gram) 100gBW (1000 gram body weight)

All values mean ± standard error of mean, * $p<0.05$, ** $p<0.01$, *** $p<0.001$ statistical comparisons were made between control and ring groups using the Student t test.

(3) Left Ventricular Hypertrophy (LVH).

Three different sized rings were tried on the ascending aorta.

(i) Ring 18 (internal diameter 1.13 mm) reduced the diameter of the aorta by approximately 55 %, leaving 20 % of its original cross sectional area patent.

(ii) Ring 17 (internal diameter 1.40 mm) reduced the diameter of the aorta by approximately 45 %, leaving approximately 30 % of its original cross sectional area patent.

(iii) Ring 16 (internal diameter 1.55 mm) reduced the diameter of the aorta by approximately 40 %, leaving roughly 37 % of the original area patent.

The aortic constriction operation had an overall mortality rate of 16 % - deaths were due to penetration of atria or vena cava during the thoracotomy. The post-operative mortality rate of Ring 18 animals was 100 %, all deaths were due to acute left ventricular failure and occurred within an hour of surgery. There were no post-operative deaths among the Ring 16 and 17 animals.

In the two weeks following the operation, rats with aortic rings regained their pre-operative weights and surpassed it by an average of 15 g.

Two weeks after placement of the rings cardiac catheterisation was attempted and the HW/BW determined, using the BW at catheterisation. Ring 17 resulted in a highly significant increase in the left ventricular weight, body weight ratio (LVW/BW) (+36.7 $\pm$ 5.4%) but no significant change in the right ventricular weight, body weight ratio (RVW/BW), the right ventricular weight, left ventricular weight ratio (RVW/LVW) was significantly decreased. Ring 16 also increased the LVW/BW significantly (+28.8 % $\pm$ 2.8%) but did not alter the RVW/BW significantly. The RVW/LVW was significantly decreased. The dry weight of the ventricles did not differ significantly from the wet weights. (See Table 2).

As Ring 17 animals were extremely difficult to catheterise and did not survive past the first infusion unit, Ring 16 animals were used in the comparative studies and will be referred to as the LVH group.

(4) Right Ventricular Hypertrophy (RVH)

Three different sized rings were tried on the pulmonary artery,

(i) Ring 16 (internal diameter 1.55 mm)

(ii) Ring 15 (internal diameter 1.73 mm) and

(iii) Ring 13 (internal diameter 2.31 mm),

these reduced the diameter of the pulmonary artery by approximately 40 %, 32 % and 9 % respectively, leaving 37 %, 46 % and 83 % of the original area patent.

When ring 13 was closed on the artery it did not appear to constrict it at all, so was not used.

The overall mortality rate for the pulmonic constriction operation was 25 %. There was only one post-operative death, caused by retention of a swab, the others took place during the thoracotomy and were from haemorrhage due to penetration of an atrium or vena cava.

The time from surgery to catheterisation of these animals ranged from 10 to 15 days. Few animals regained their pre-operative weights by the time that they were catheterised and the majority had lost weight, the average weight loss was 20 g in those with Ring 16 and 15. The HW/BWs were determined using the BW at catheterisation.

Ring 16 produced a significant increase ($p < 0.01$) in the RVW/Bw (+39.0 % $\pm$ 11.6%) and a significant decrease in ($p < 0.05$) in the LVW/BW (16.2 % $\pm$ 2.2 %). The RVW/LVW was significantly increased. All but one of these nimals had developed large paper thin fibrotic areas in the free wall of the outflow tract of the right ventricle and in the region immediately underneath it. The fibrotic areas increased in size as the

period of ring application increased and, as well as reducing the mean ventricular weight, they contributed to the wide scatter of results. The only rats with no visible signs of fibrosis (one killed after 10 days of constriction) had a right ventricular hypertrophy of 72.1 %, while one with the largest fibrotic area (10 x 15 mm), one killed after 14 days of constriction, had a ventricular weight increase of only 7.1 %. The changes in ventricular dry weights did not differ significantly from those seen with the wet weights.

Ring 15 produced a significant increase in the RVW/BW (+51.1 % ± 6.8 %) and a non-significant decrease in the LVW/BW (-6.8 % ± 2.3 %). The RVW/LVW was significantly increased. Although Ring 15 resulted in a less severe constriction than Ring 16 it produced a higher percentage increase in RVW/BW as fibrosis was less frequent and less extensive when found. The largest fibrotic area found in a Ring 15 rat was approximately 6 x 6 mm, it was found after 14 days of constriction but only reduced the percentage hypertrophy to 32.8 %. The changes in ventricular dry weights did not differ from those seen with ventricular wet weights.

As there was no significant difference in the ventricular weights of rats with RVH due to Ring 15 or 16 these results were combined for comparative purposes into a RVH group with a mean increase in RVW/BW of 44.5 % ± 6.1 % and a mean decrease in LVW/BW of 10.03 ± 2.0 %, with a RVW/LVW significantly above normal. The percentage increase in right ventricular weight in this group was 16 % higher than the increase in left ventricular weight in the LVH group. The RVH group was then redivided into two sub-groups, one with high hypertrophy (62.0 % ± 6.5 %) and one with low hypertrophy (31.9 % ± 1.7%), comparable to that seen in the LVH group, and their performances compared. Although the ventricular weights of these sub-groups were significantly different ($p < 0.05$), their basal and post-infusion cardiac output and pressure measurements did not differ significantly; so it was concluded that it would be valid to compare the original RVH group and the LVH group, despite the difference in degree of hypertrophy.

Basal Values of all Parameters before Infusion - Table 3

Group	Cardiac Output (ml/min)	Cardiac Output (ml/min/Kg)	Heart Rate (beats/min)	Femoral Artery Press.	RVSP (mm Hg)	RVEDP (mm Hg)	LVSP (mm Hg)	LVEDP (mm Hg)
NORMAL (14)	89.9 ± 10.9	196.3 ± 24.2	385 ± 6	140.8 ± 3.9	33.2 ± 1.9	0.1 ± 3.0	166.9 ± 6.7	1.4 ± 0.4
11 SHAM (6)	NS 92.4 ± 15.5	NS 189.2 ± 29.1	NS 360 ± 18	NS 138.9 ± 10.0	NS 29.6 ± 3.6	NS 0.2 ± 0.5	NS 153.3 ± 12.4	NS 1.0 ± 0.3
6 LVH (7) 8	NS 80.4 ± 9.6	NS 162.0 ± 19.0	NS 393 ± 7	NS 128.8 ± 8.4	NS 30.8 ± 4.6	NS 1.1 ± 0.5	*** 243.0 ± 18.8	* 4.0 ± 1.5
10 RVH (10) 10	NS 92.0 ± 15.9	NS 175.0 ± 17.7	*** 326 ± 14	*** 113.9 ± 3.9	** 48.8 ± 4.3	NS 0.7 ± 0.8	NS 138.3 ± 9.3	NS 1.8 ± 1.0

NB Mean ± Standard error of Mean: *$p<0.05$, **$p<0.01$, ***$p<0.001$ statistical comparisons were made between normal and other groups using the Student t test.

(n) number of rats in which the intraventricular pressures were measured.

n number of rats in which cardiac outputs were measured.

RVH rats with right ventricular hypertrophy due to pulmonary artery ring 15 or 16.

LVH rats with left ventricular hypertrophy due to aortic ring 16.

RVSP right ventricular systolic pressure

RVEDP right ventricular end-diastolic pressure

LVSP left ventricular systolic pressure

LVEDP left ventricular end-diastolic pressure.

B. Cardiac Output (Table 2)

(1) Normal

The cardiac output of the normal group averaged 89.9 ± 10.9 ml/min., or expressed as per Kg BW as, 196.3 ± 24.2 ml/min/Kg. The basal stroke volume was 51.4 ± 6.5 x 10^{-2} ml/Kg (Heart rate 385 ± 6 beats per minute). As these results were obtained by the direct Fick Method, which measures the cardiac output of the right ventricle, the stroke volume was also expressed per gram of right ventricular tissue, and was 164.0 ± 17.0 x 10^{-2} ml/Kg/gRV.

(2) Sham

The basal cardiac output of the sham operated group was 92.4 ± 15.5 ml/min or 189.2 ± 29.1 ml/min/Kg. The basal stroke volume was 51.6 ± 6.3 x 10^{-2} ml/Kg (Heart rate 360 ± 18 beats per minute) or 151.1 ± 18.3 x 10^{-2} ml/Kg/gRV. None of these results differed significantly from those of the normal group.

(3) LVH

The basal cardiac output of the LVH group was 80.4 ± 9.6 ml/min or 162.0 ± 19.0 ml/min/Kg. The stroke volume was 42.0 ± 4.5 x 10^{-2} ml/Kg (heart rate 393 ± 7 beats per minute) or 131.0 ± 13.6 x 10^{-2} ml/Kg/gRV. None of these values differed significantly from those of the normal group.

(4) RVH

The basal cardiac output of the RVH group was 92.0 ± 15.9 ml/min or 175.0 ± 17.7 ml/min/Kg, values which were not significantly different from those of the normal group. Though the heart rate of the RVH group was significantly lower than the normal value, the stroke volume (52.7 ± 5.6 x 10^{-2} ml/Kg) did not differ significantly from the normal group. However when the stroke volume was expressed per gram RV tissue (118.2 ± 11.4 x 10^{-2} ml/Kg/gRV) it was significantly below normal.

C. Other Parameters

(1) Left Side Pressures

The frequency response of the catheter manometer system used to record the left ventricular pressures was 29 cps.

Left Side Pressures (Normal Rat) Pressure Tracing 1)

Paper speed 80.5 mm/sec.

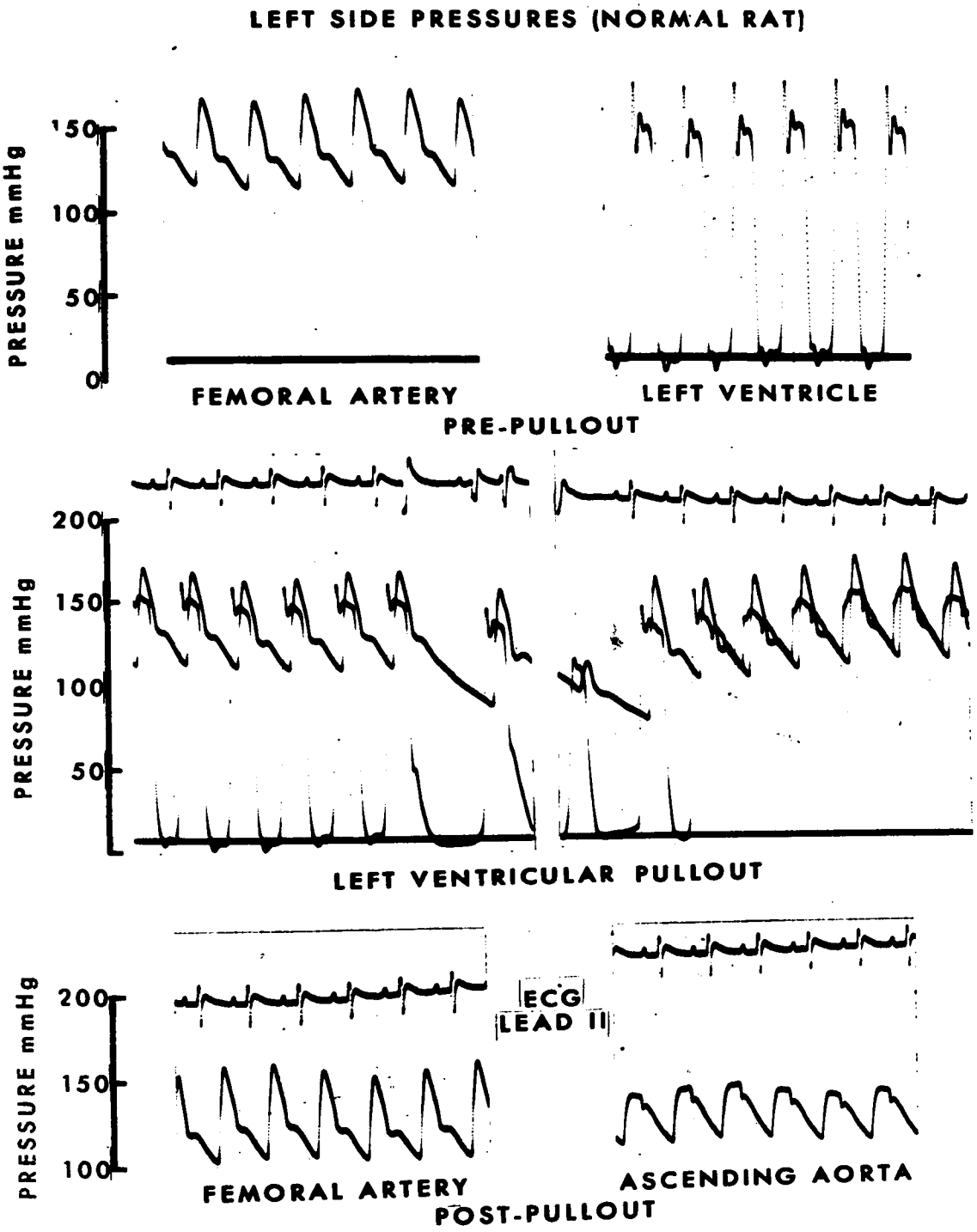
All pulse pressures were recorded by intravascular catheterisation.

Note artefact at peak of left ventricular pressure pulse.

Left Ventricular Pullout - continuous pressure recording made while cathether is being withdrawn from the left ventricle, through the aortic valve and into the ascending aorta. In the gap in this recording 6 ectopic beats were cut out.

ECG Electrocardiogram.

PRESSURE TRACING I



Left Ventricular Pullout (Normal Rat) (Pressure Tracing 2)

Paper speed 80.5 mm/sec.

LV left ventricular pressure

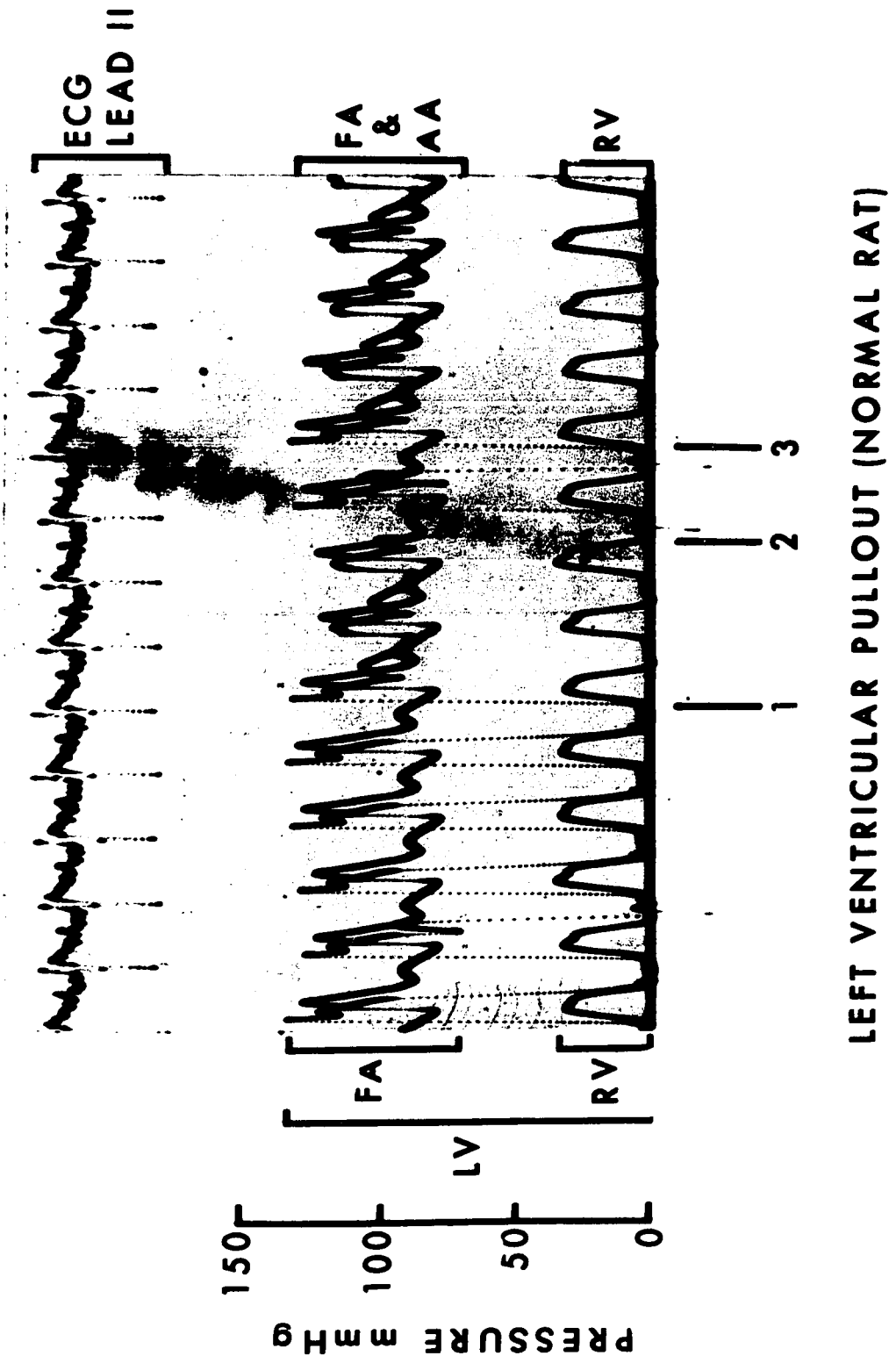
FA Femoral artery pressure

RV right ventricular pressure

AA pressure in ascending aorta.

1. LV catheter flips through aortic valve into aorta.
2. LV catheter flips back into the left ventricle for 2 beats.
3. LV catheter returns to ascending aorta.

ECG electrocardiogram.



LEFT VENTRICULAR PULLOUT (NORMAL RAT)

PRESSURE TRACING 2

(a) Normal

12 % of catheterisations were unsuccessful; these took place early in the study and were due to general lack of experience rather than any specific reason.

The left ventricular peak systolic pressure (LVSP) was 166.9 ± 6.7 mm Hg and the left ventricular end-diastolic pressure (LVEDP) was 1.4 ± 0.4 mm Hg. The mean femoral artery pressure was 140.8 ± 3.9 mm Hg.

Normal LV pullouts (continuous pressure recordings made while the catheter is being withdrawn from the ventricle) exhibited a drop in peak systolic pressure of 2-10 mm Hg (the average drop being 5 mm Hg) across the valve. The systolic femoral artery pressure was 15-20 mm Hg above the systolic pressure in the ascending aorta (see pressure tracings 1 and 2).

(b) Sham

All sham operated rats were catheterised successfully.

The LVSP was 153.3 ± 12.3 mm Hg and the LVEDP was 1.0 ± 0.3 mm Hg. The femoral artery pressure was 138.9 ± 10.0 mm Hg. None of these results differed significantly from those of the normal group.

(c) LVH

45 % of catheterisations of rats with aortic rings were unsuccessful. This high failure rate was due to two factors:-

1. Placement of the ring too close to the carotid artery (see Illustration 1).
2. The problems encountered with the Ring 17 group. The hypertrophied ventricles of rats in this group were hyperirritable and touching the ventricular wall with the catheter resulted in fatal tachycardiac in 2 rats while the intraventricular pressure

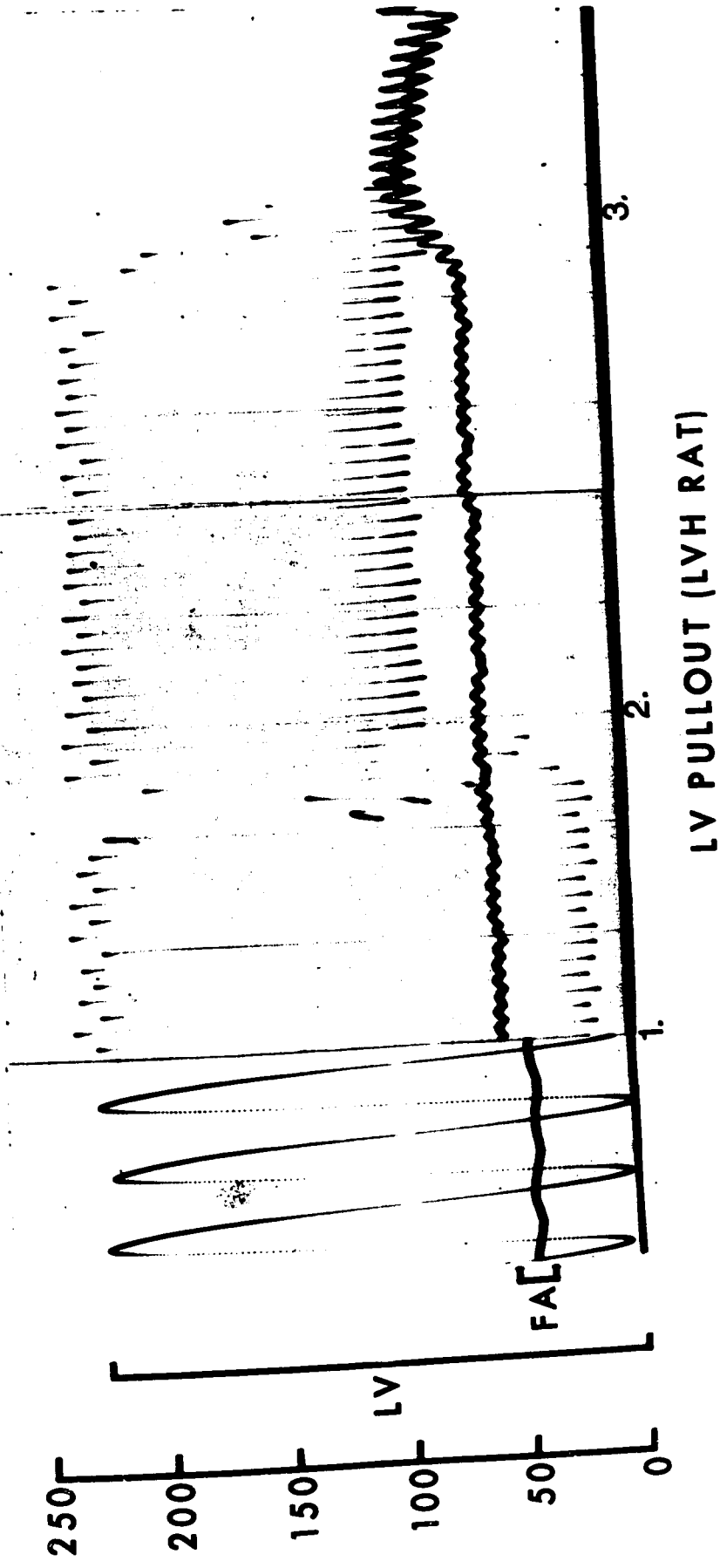
LV Pullout (LVH Rat) (Pressure Tracing 3)

LV left ventricular pulse pressure.

FA femoral artery pulse pressure.

1. Paper speed initially 80.5 mm/sec, then reduced to 19.5 mm/sec.
2. The LV catheter was drawn from the LV, through the aortic valve and into the supravalvular chamber (between the valve and the ring) in which the systolic pressure equalled that of the LV and the diastolic was the same as that of the ascending aorta beyond the ring. A short section of the supravalvular recording was cut out of the centre of the pressure tracing, so it would fit onto the page - the missing section did not differ from the rest of the supravalvular trace.
3. The LV catheter was then drawn through the aortic ring and into the ascending aorta.

Note that the LV pressure pulse exhibited the peaking typical of a dynamically significant degree of stenosis and that the femoral artery pulse pressure was very damped but less so after the catheter had been withdrawn from the ring.



PRESSURE TRACING 3

LV Pullout After IU 2 (LVH Rat) (Pressure Tracing 4)

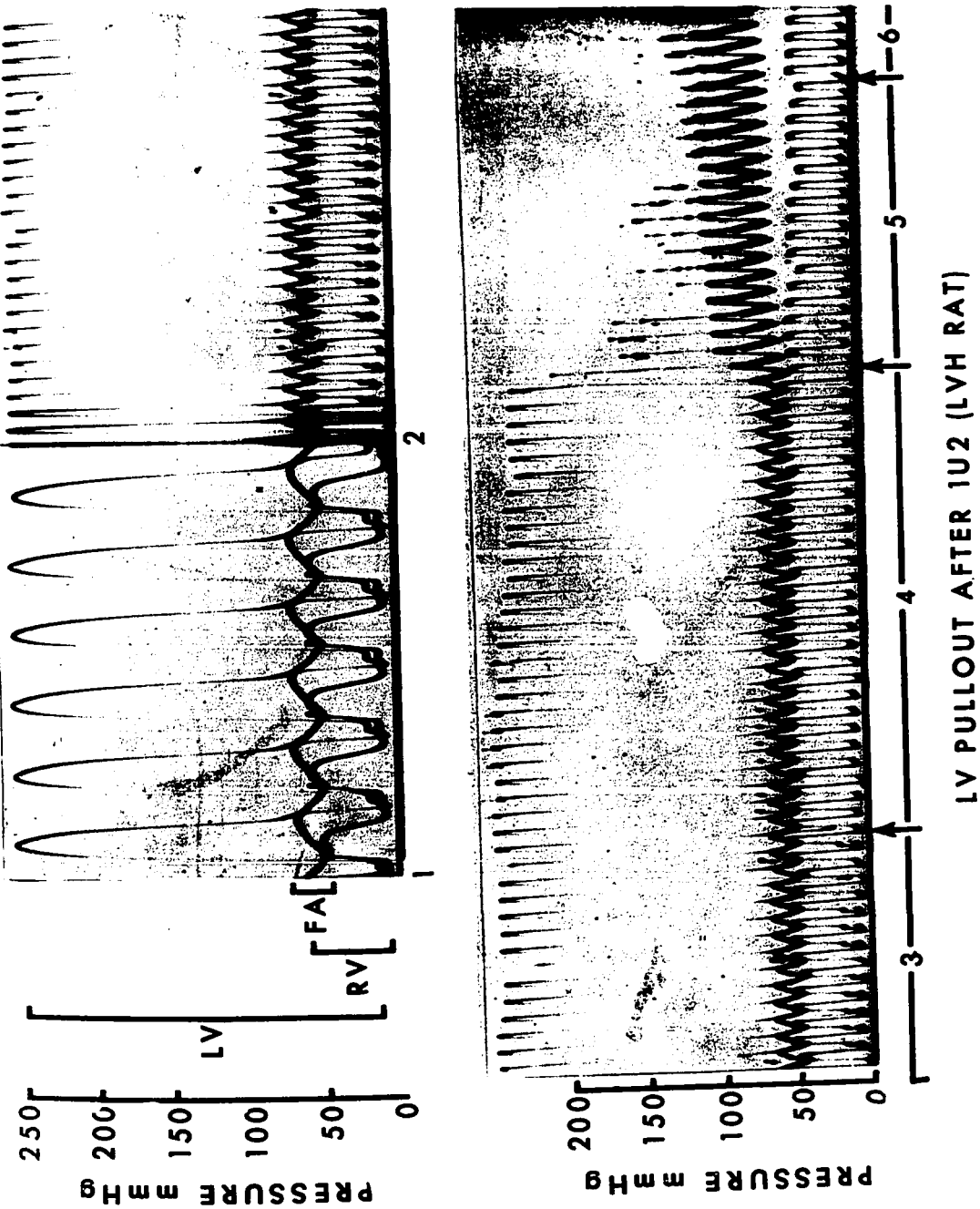
LV left ventricular pressure.

RV right ventricular pressure.

FA femoral artery pressure.

1. Paper speed 80.5 mm/sec.
2. Paper speed 19.5 mm/sec.
3. LV catheter in LV, at arrow flips through aortic valve.
4. LV catheter in supravalvular chamber, between valve and ring, at arrow flips through ring.
5. LV catheter in ascending aorta.
6. LV catheter in left carotid artery.

Note peaking of LV trace and damping of FA pulse pressure which is reduced after the catheter is withdrawn from the ring.



PRESSURE TRACING 4

(c) LVH (Continued)

2. (Cont'd)

was so high in another that it continually forced the catheter out of the ventricle and efforts to replace it lead to the animal's death. None of the three animals catheterised survived for more than one infusion unit, so only rats with LVH due to constriction with ring 16 were used in comparative studies with normal rats and with those with RVH.

The LVSP of the LVH group was 243.0 ± 18.8 mm Hg, which was significantly higher ($p < 0.001$) than the normal value and the LVEDP was 4.0 ± 1.5 mm Hg was significantly ($p < 0.05$) above normal. However, the mean femoral artery pressure, 128.8 ± 8.4 mm Hg, did not differ significantly from normal.

Pressure tracings from the hypertrophied left ventricle were more peaked than those obtained from normal ventricles.

The left ventricular pullouts clearly showed a transitional zone between the aortic valve and the constricting ring in which the systolic pressure equalled that of the left ventricle and the diastolic pressure was the same as that in the ascending aorta beyond the ring. The systolic pressure gradients beyond the ring averaged 100-120 mm Hg. In some cases the femoral artery pulse pressure was less damped when the catheter was pulled from inside the ring. (See pressure tracings 3 and 4).

(d) RVH

The LVSP of the RVH group was 138.3 ± 9.3 mm Hg and the LVEDP was 1.8 ± 1.0 mm Hg, neither of these results differed significantly from normal. However the mean femoral artery pressure was significantly ($p < 0.001$) below that of the normal group.

The LV pressure tracing from this group was very similar in shape to that of the normal group, while the systolic gradient was of the order of 2-7 mm Hg.

LV Pullout (RVH Rat) (Pressure Tracing 5)

Paper speed 80.5 mm/sec.

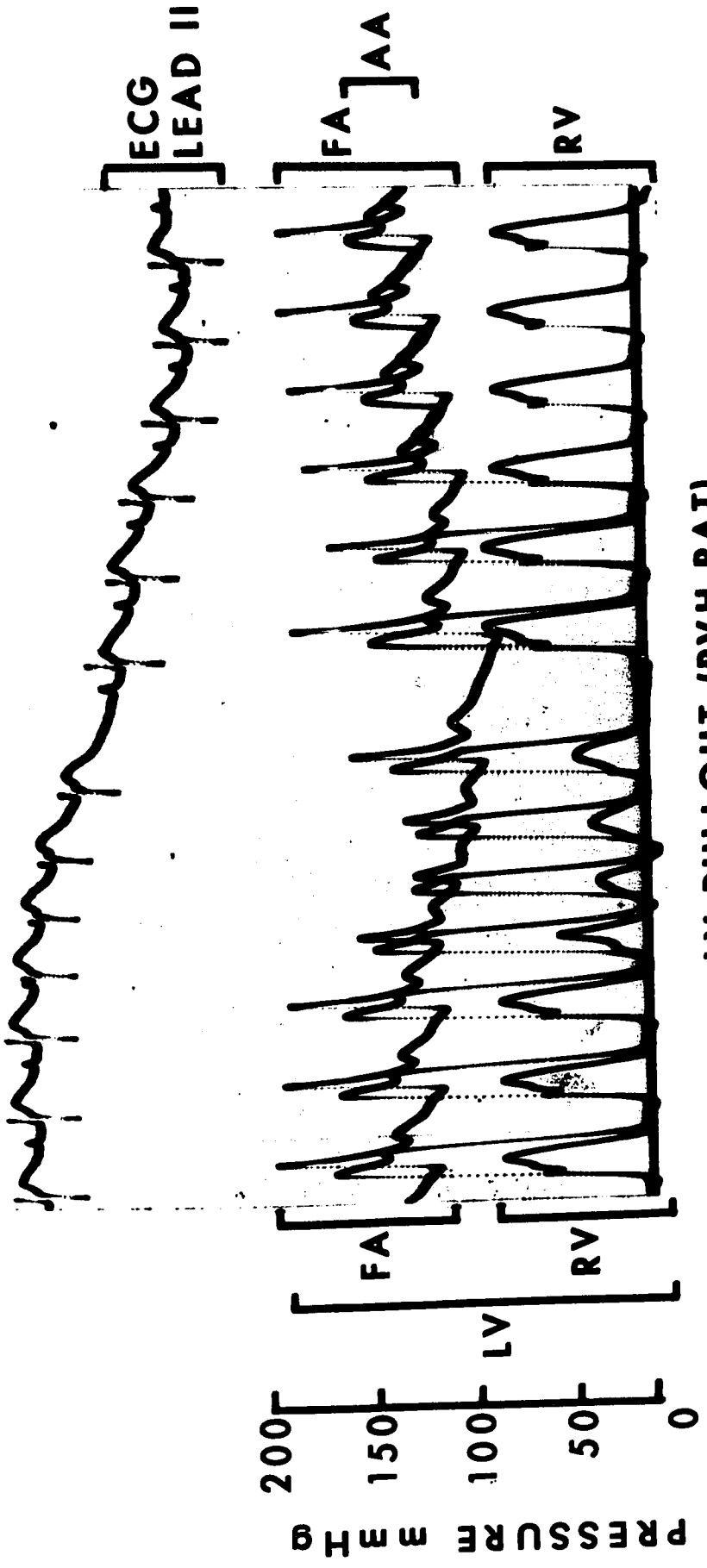
LV left ventricular pressure pulse.

RV right ventricular pressure pulse, note peaking typical of a dynamically significant degree of stenosis.

FA femoral artery pressure pulse.

AA ascending aorta pressure.

ECG electrocardiogram.



PRESSURE TRACING 5

(2) Right Sided Pressures

The frequency response of the right ventricular catheter transducer system was 34 cps.

(a) Normal

The RVSP of the normal group was 33.3 ± 1.9 mm Hg and the RVEDP was 1.0 ± 3.0 mm Hg.

A clear tracing from a normal right ventricle may be seen on pressure tracing 2.

(b) Sham

The RVSP of the sham operated group was 29.6 ± 3.6 mm Hg and the RVEDP was 0.2 ± 0.5 mm Hg, neither of these results were significantly different from normal.

(c) LVH

The RVSP of the LVH group was 30.8 ± 4.6 mm Hg and the RVEDP was 1.1 ± 0.5 mm Hg, neither of these results differed significantly from normal.

The RV pressure tracing appeared similar in contour to that of the normal group. In some cases in the RVSP decreased when the catheter was pulled out from inside the ring. (See pressure tracing 4).

(d) RVH

22 % of catheterisations of rats with pulmonary artery rings were unsuccessful, these failures were due to the difficulty of catheterising the elarged RV. The majority of unsuccessful catheterisations were in animals with Ring 16. In these rats the right atrium had undergone such gross hypertrophy that the usual manipulative procedures resulted in the catheter catching in the atrial wall rather than entering the ventricle.

The RVSP of 48.8 ± 4.3 mm Hg was significantly above ($p < 0.01$) the normal value but the RVEDP of 0.7 ± 0.8 mm Hg did not differ significantly from normal.

The RV pressure tracing from the hypertrophied ventricles were more pointed than those from normal right ventricles (see pressure tracing 5).

(3) Stroke Work

NB Stroke work was calculated using cardiac output per Kg BW.

(a) Normal

The basal stroke work of the normal group was 9.77 ± 1.22 x 10^{-3} Kg M.

(b) Sham

The basal stroke work in the sham operated group was 10.98 ± 1.69 x 10^{-3} Kg M, which was not significantly different from that of the normal group.

(c) LVH

The basal stroke work of the LVH group was 8.82 ± 1.41 x 10^{-3} Kg M, which was not significantly different from that of the normal group.

(d) RVH

The basal stroke work of the RVH group was 8.74 ± 1.11 x 10^{-3} Kg M, which was not significantly different from that of the normal group.

Right Ventricular Developed Pressure in mm Hg - Table 4

Group	Basal	IU 1	IU 2	IU 3	IU 4	IU 5	IU 6	IU 7
Normal	33.1 ± 1.9	33.1 ± 1.6	31.2 ± 1.6	27.4 ± 2.3	21.8 ± 2.2	19.0 ± 3.4	16.9 ± 4.0	17.2 ± 4.1
	NS	NS	*	NS	NS			
LVH	29.7 ± 4.7	38.8 ± 5.4	40.4 ± 3.5	29.0 ± 5.6	18.5 ± 5.2	(14.3)		
	**	***	**	**	NS	NS		
RVH	48.1 ± 4.8	55.1 ± 3.0	48.9 ± 5.1	45.1 ± 4.1	31.0 ± 5.1	21.1 ± 4.3		

Left Ventricular Developed Pressure in mm Hg

Group	Basal	IU 1	IU 2	IU 3	IU 4	IU 5	IU 6	IU 7
Normal	165.5 ± 6.7	172.2 ± 6.6	130.0 ± 3.5	110.9 ± 3.5	102.0 ± 6.1	98.3 ± 10.6	86.4 ± 12.6	87.0 ± 4.4
	*	***	*	NS	NS			
LVH	239.0 ± 19.2	258.8 ± 14.8	169.4 ± 18.4	123.6 ± 20.7	90.7 ± 20.7	(67.3)		
	*	**	*	NS	NS	NS		
RVH	136.5 ± 9.6	137.0 ± 7.1	110.4 ± 7.9	101.9 ± 3.9	80.2 ± 6.8	69.2 ± 9.5	(30.8)	

Normal (14 rats) LVH left ventricular hypertrophy (7 rats) RVH right ventricular hypertrophy (10 rats) IU infusion unit.

All values mean ± standard error of mean, ( ) number of rats if less than 5.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Statistical comparisons were made between the normal and hypertrophy groups using the Student t test.

(4) Developed Pressure. (Table 4)

NB Developed Pressure (DP) = peak systolic pressure - end-diastolic pressure.

(a) Normal

The right ventricular developed pressure was 33.1 ± 1.9 mm Hg and the left ventricular developed pressure was 165.5 ± 6.7 mm Hg.

(b) Sham

The right ventricular developed pressure was 30.4 ± 1.4 mm Hg and the left ventricular developed pressure

Neither of these results were significantly different from normal.

(c) LVH

The RVDP was 29.7 ± 4.8 mm Hg, which was not significantly different from normal. The LVDP was 239.0 ± 19.2 mm Hg, which was significantly higher than normal.

(d) RVH

The RVDP was 48.1 ± 4.8 mm Hg, which was significantly higher than normal. The LVDP was 136.5 ± 9.6 mm Hg, which did not differ significantly from normal.

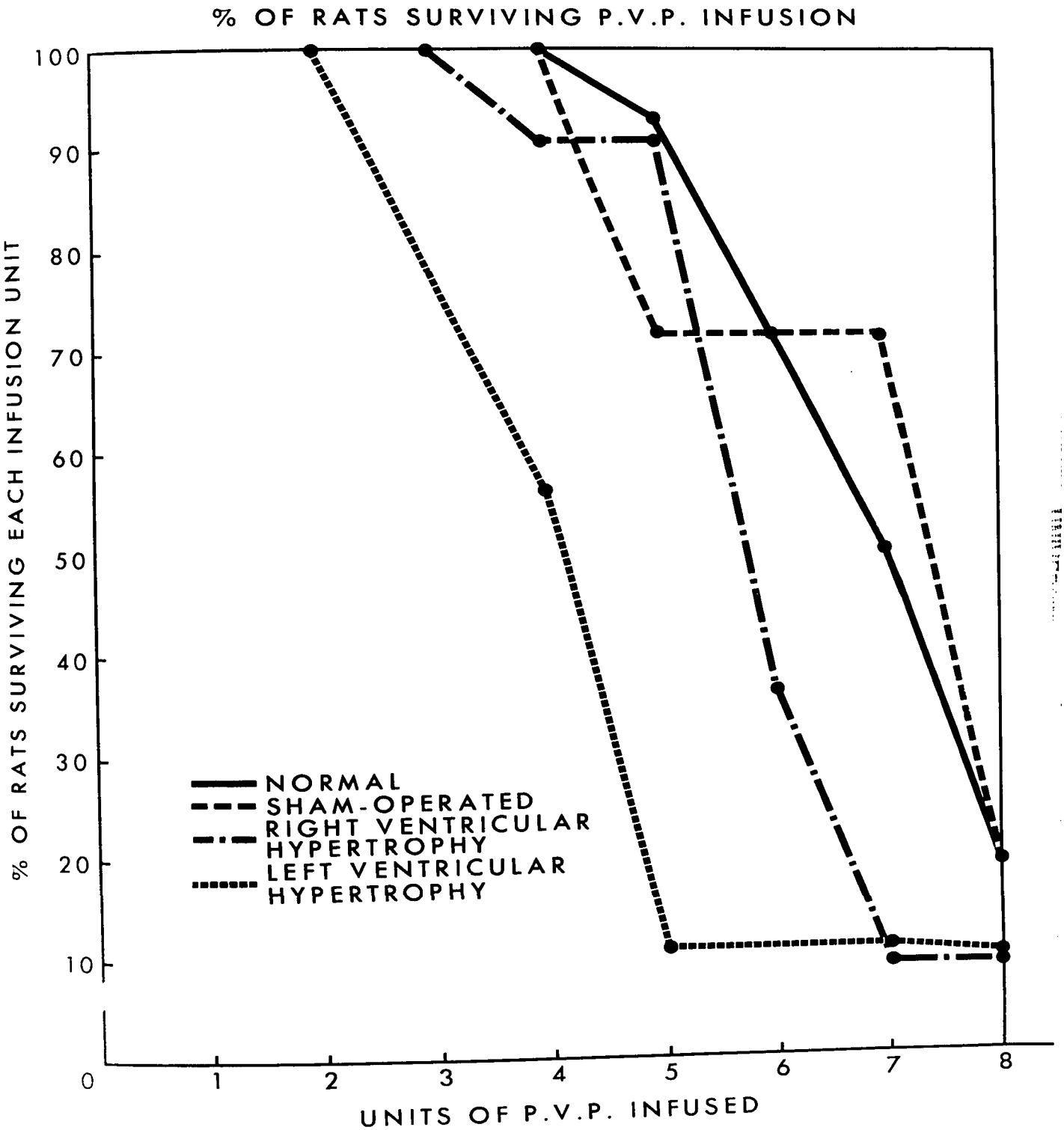

% OF RATS SURVIVING P.V.P. INFUSION
% OF RATS SURVIVING EACH INFUSION UNIT
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
UNITS OF P.V.P. INFUSED
NORMAL
SHAM-OPERATED
RIGHT VENTRICULAR HYPERTROPHY
LEFT VENTRICULAR HYPERTROPHY

Figure 2

D. Changes During Infusion

(1) Survival rates. (See Figure 2)

(a) Normal and Sham

Most of the normal and sham operated rats survived for 6 infusion units (IU) and at least half for 7 IU.

(b) LVH

The survival rate was lowest in the LVH group, just over half (56%) lasting for 4 IU and only one rat surviving for any longer.

(c) RVH

Most of the RVH group survived for 5 IU, while less than half survived for 6 IU and only one rat survived any longer.

(2) Cardiac Output

(a) Normal

Figure 3: The cardiac output (ml/min/Kg) increased rapidly during infusion unit 1 - more than doubling its basal value. It rose more slowly during the next 4 infusion units, reaching a maximum value at post-infusion measuring period 4. (PMP 4). During IU 5 the cardiac output decreased slightly and fell more rapidly in IU 6 - this later result was biased as it represented only 3 rats.

Figure 4: The changes in stroke volume per gram of right ventricular tissue followed a similar pattern to the cardiac output. The stroke volume rose steeply during the first infusion unit and then more slowly, reaching a peak at PMP 4.

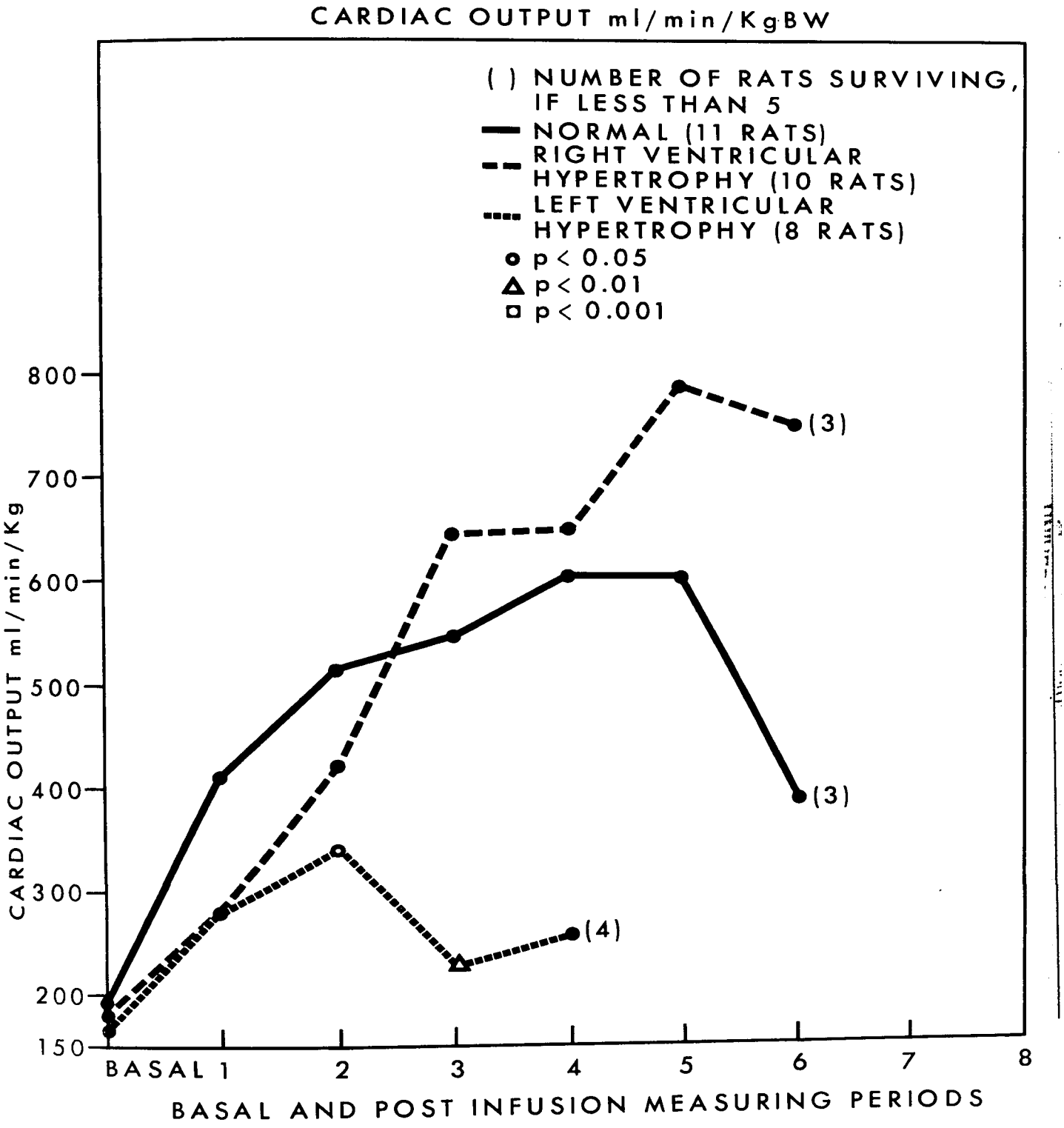

CARDIAC OUTPUT ml/min/KgBW
( ) NUMBER OF RATS SURVIVING, IF LESS THAN 5
NORMAL (11 RATS)
RIGHT VENTRICULAR HYPERTROPHY (10 RATS)
LEFT VENTRICULAR HYPERTROPHY (8 RATS)
p < 0.05
p < 0.01
p < 0.001
800
700
600
500
400
300
200
150
CARDIAC OUTPUT ml/min/Kg
(3)
(3)
(4)
BASAL 1
2
3
4
5
6
7
8
BASAL AND POST INFUSION MEASURING PERIODS

Figure 3

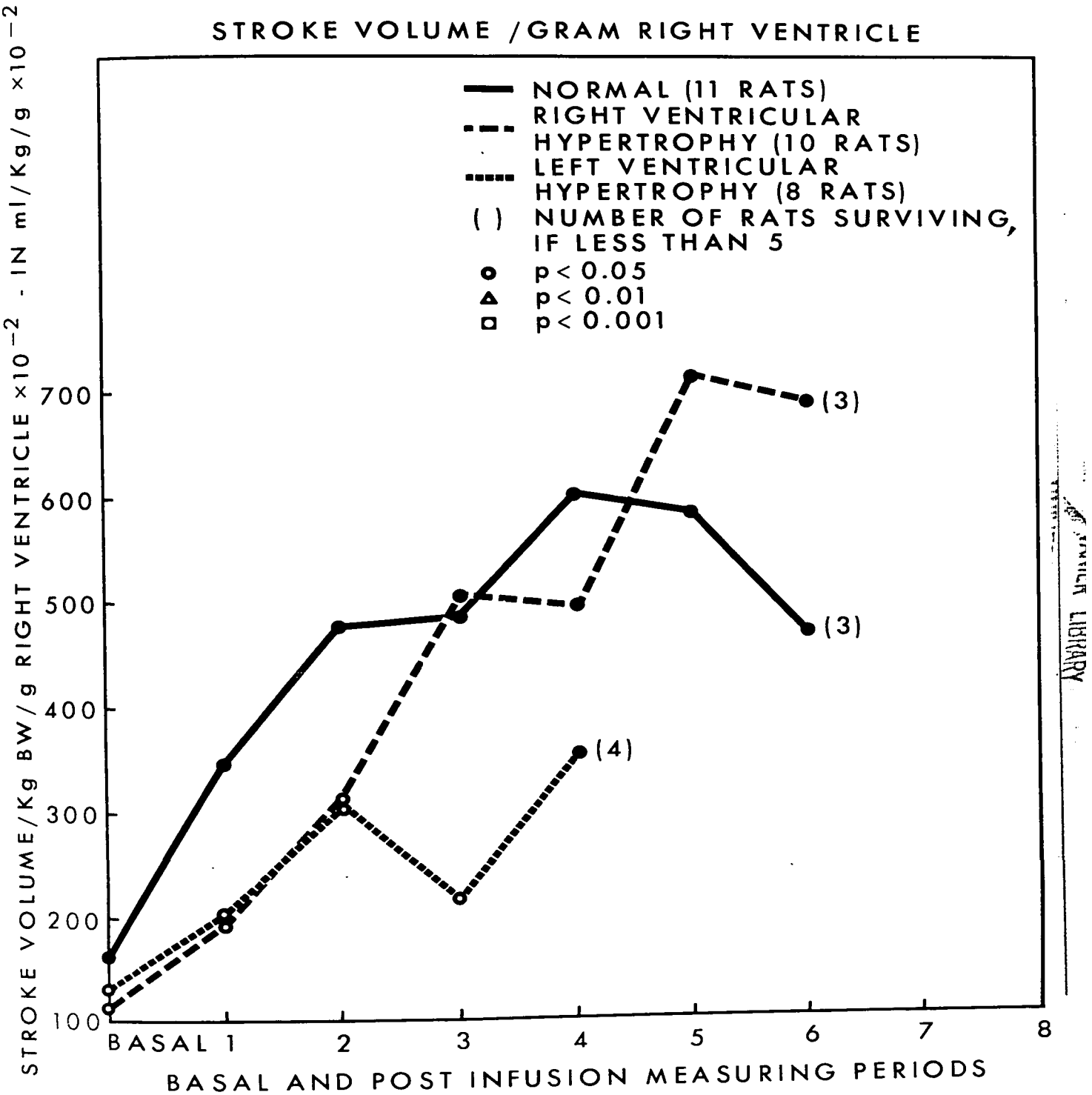


Figure 4

(b) Sham

The changes in stroke volume and cardiac output of the sham operated group did not differ significantly from normal and were not included on these figures.

(c) LVH

Figure 3: The cardiac output (ml/min/Kg) rose to a maximum value at PMP 2. However this value was significantly lower ($p<0.05$) than the normal value at this period. It then fell to a value still significantly ($p<0.01$) below the normal value at PMP 3 and rose very slightly in IU 4, but only 4 rats survived to PMP 4.

Figure 4: The stroke volume per gram right ventricular tissue rose to a maximum at PMP 1 and then fell again, however at all post-infusion measuring periods it was significantly below the normal values.

(d) RVH

Figure 3: The cardiac output (ml/min/Kg) rose to a peak at PMP 3, declined slightly during IU 4 and then rose to a second and higher peak during IU 5. At no stage did the values differ from those of the normal group.

Figure 4: The stroke volume per gram RV tissue rose slowly during the first two infusion units, both the values at PMP 1 and PMP 2 were significantly below normal. However by PMP 3 it had climbed back into the normal range, peaking at PMP 3 and 5 as did the cardiac output.

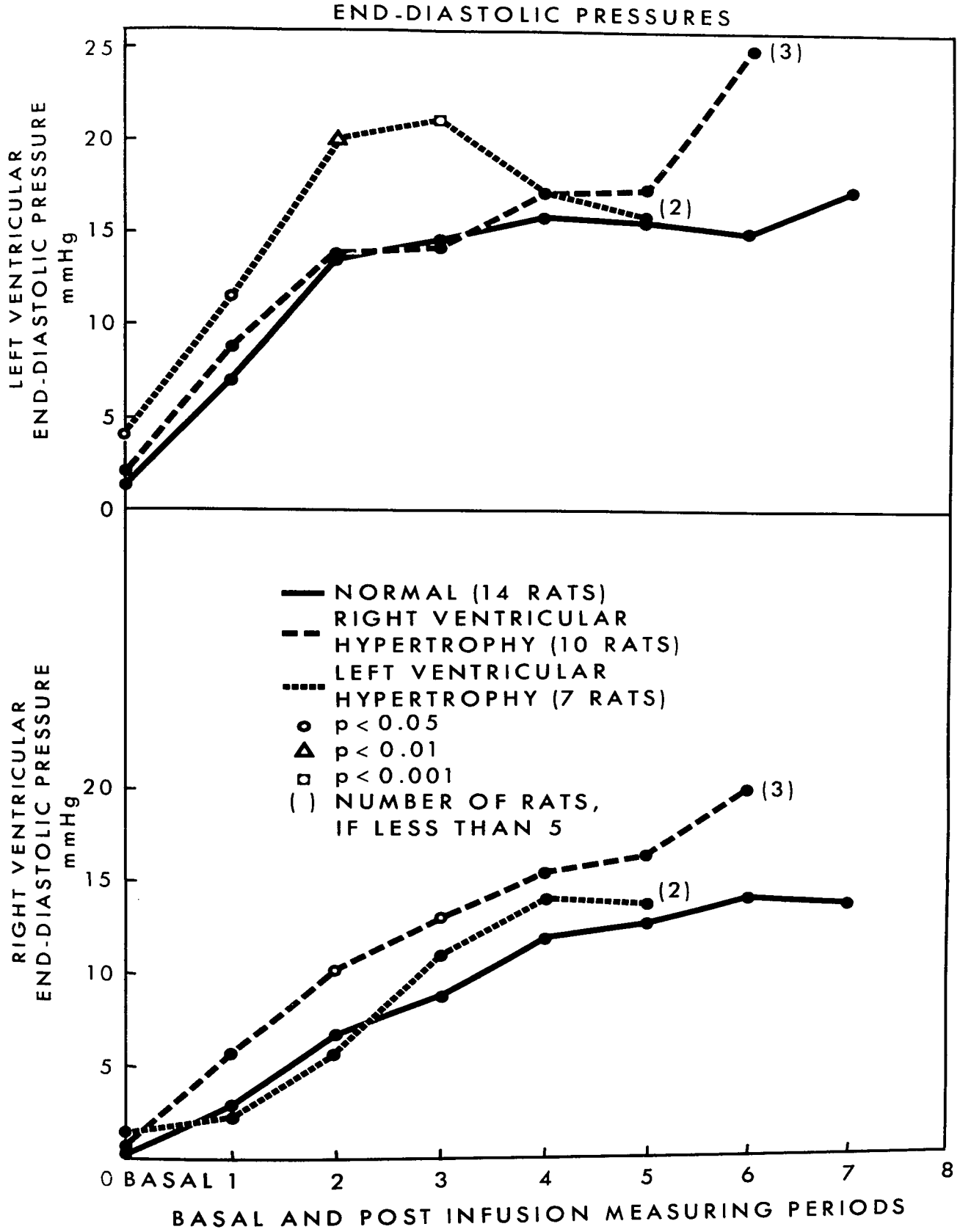

END-DIASTOLIC PRESSURES
LEFT VENTRICULAR END-DIASTOLIC PRESSURE mmHg
RIGHT VENTRICULAR END-DIASTOLIC PRESSURE mmHg
25
20
15
10
5
0
(3)
(2)
NORMAL (14 RATS)
RIGHT VENTRICULAR HYPERTROPHY (10 RATS)
LEFT VENTRICULAR HYPERTROPHY (7 RATS)
o p < 0.05
Δ p < 0.01
□ p < 0.001
( ) NUMBER OF RATS, IF LESS THAN 5
0 BASAL 1 2 3 4 5 6 7 8
BASAL AND POST INFUSION MEASURING PERIODS

Figure 5

(3) Left Side Pressures (Figures 5, 6 and 7)

Figure 5: Continuous monitoring showed that when the infusion pump was turned on the LVEDP rose steadily until the pump was stopped and then fell steadily until the pump was started again. This was true for all groups and was not included on this graph.

(a) Normal

Figure 5: The normal LVEDP rose steeply for the first two infusion units and then flattened out - increasing by only 2 mm Hg in the course of the next two infusion units and then falling by 1 mm Hg during the next two.

Figure 6: The LVSP rose during the first infusion unit and then fell steadily with each succeeding unit.

Figure 7: In this graph percentage differences in systolic pressure, between the normal and each of the hypertrophy groups, at every PMP are shown. The normal values are therefore represented by the line at 0 %.

(b) Sham

None of the PMP measurements of the sham operated group were significantly different from those of the normal group, so they were not included on these graphs.

(c) LVH

Figure 5: The LVEDP rose to a maximum at PMP. The first 3 post infusion values were all significantly above normal but succeeding values were within the normal range.

PEAK SYSTOLIC PRESSURES

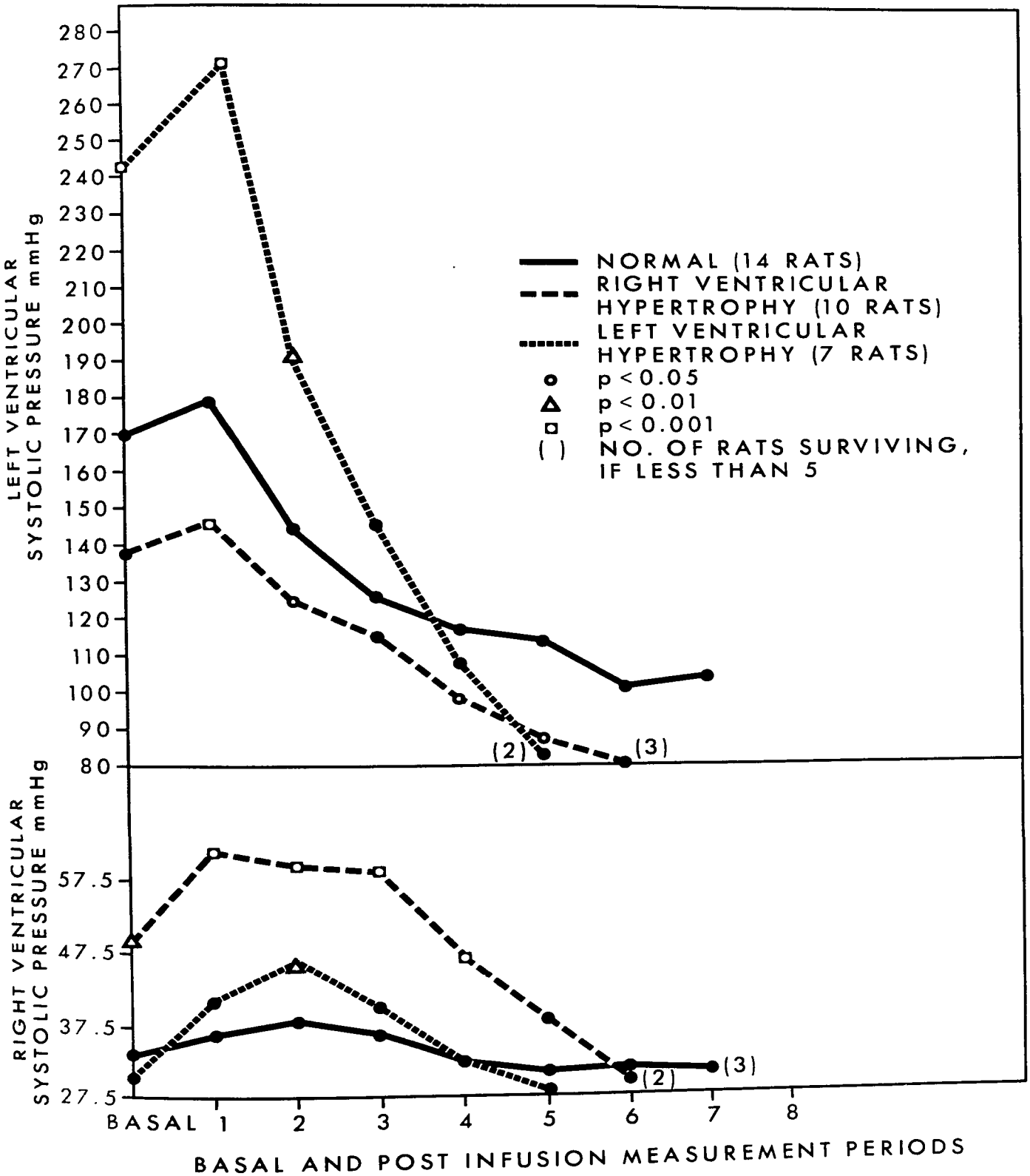


Figure 6

Figure 6: The LVSP increased during the first infusion unit to a value significantly above normal and then fell precipitously with each successive infusion unit.

Figure 7: This graph exhibited the same pattern as that seen in graph 5.

(d) RVH

Figure 5: The LVEDP of this group changed in a very similar manner to that of the normal group throughout the infusion. None of the PMP values differed significantly from the normal values.

Figure 6: The LVSP rose during the first infusion unit and then fell with each succeeding infusion unit, it resembled the normal curve but was always below it. At PMPs 1, 2, 4 and 5 its values were significantly lower than the normal group.

Figure 7: The LVSP of this group was represented by the lowest trace on this graph; it was the only one which was negative throughout the infusion.

(4) Right Side Pressures (Figures 5, 6 and 7)

Continuous pressure monitoring showed that the RVEDP, of each group, rose during each infusion unit and fell during each PMP.

(a) Normal

Figure 5: The RVEDP rose evenly with infusion unit.

Figure 6: The RVSP rose to a maximum at PMP 2 and then fell steadily for the next three infusion units.

Figure 7: This graph demonstrates the percentage difference in systolic pressure between the normal and each of the hypertrophy groups at different levels of infusion. The normal right side values are therefore represented by 0 %.

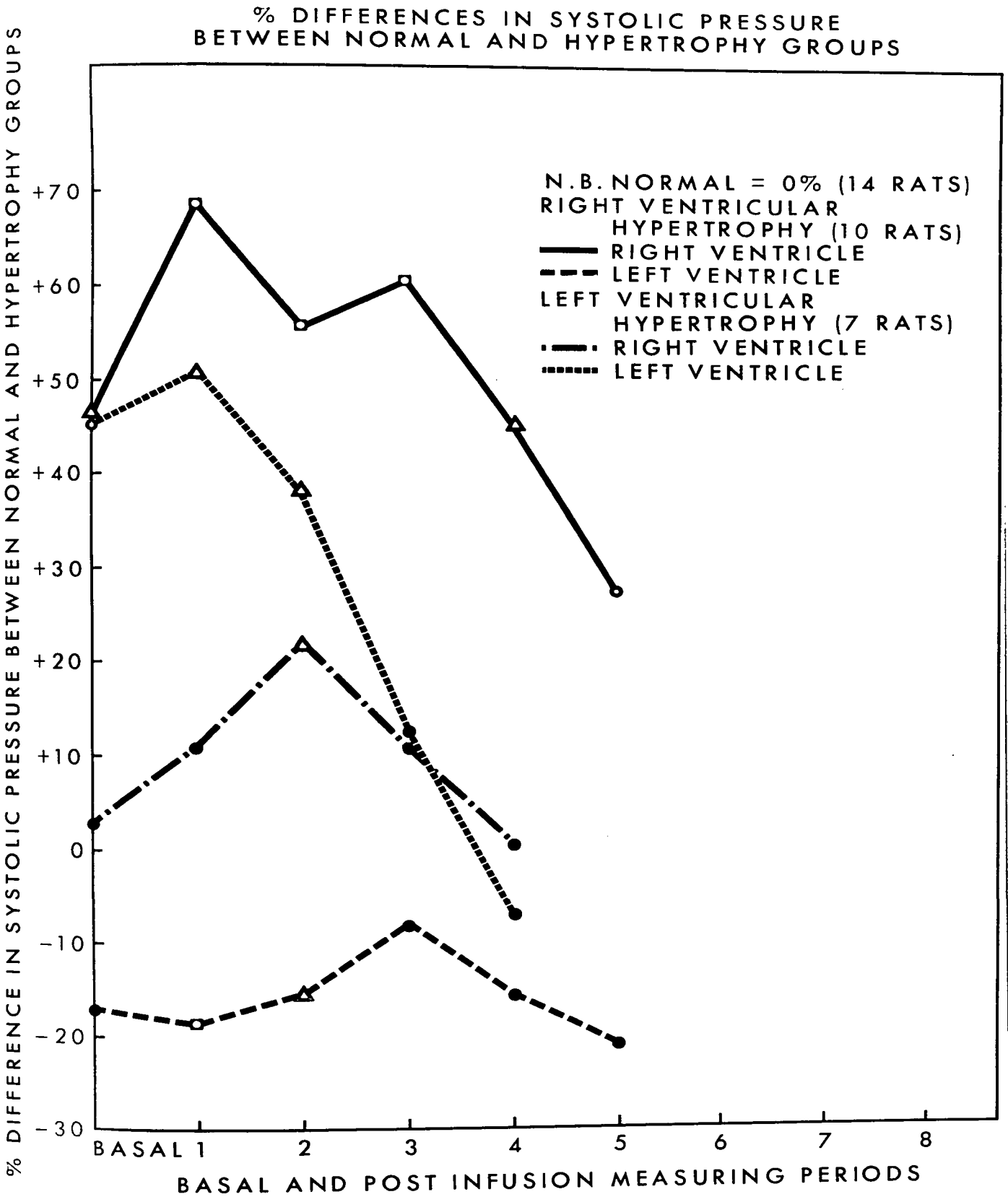


Figure 7

(b) Sham

None of the PMP measurements of this group differed from those of the normal group, so were not included on these graphs.

(c) LVH

Figure 5: The RVEDP of this group rose in a manner similar to that of the normal group - none of the PMP values differed significantly from normal.

Figure 6: The RVSP rose to a maximum value at PMP 2 which was significantly higher than normal and then fell steadily for the next three infusion units.

Figure 7: The percentage difference in systolic pressure was always positive.

(d) RVH

Figure 5: The RVEDP rose in a similar manner to that of the normal group but its values were always higher, being significantly so at PMP 2 and 3.

Figure 6: The RVSP remained significantly above the normal group for the first four infusion units.

Figure 7: The percentage differences in systolic pressure of between the RV of the RVH group and the RV of the normal group were the largest of any group and always significantly positive.

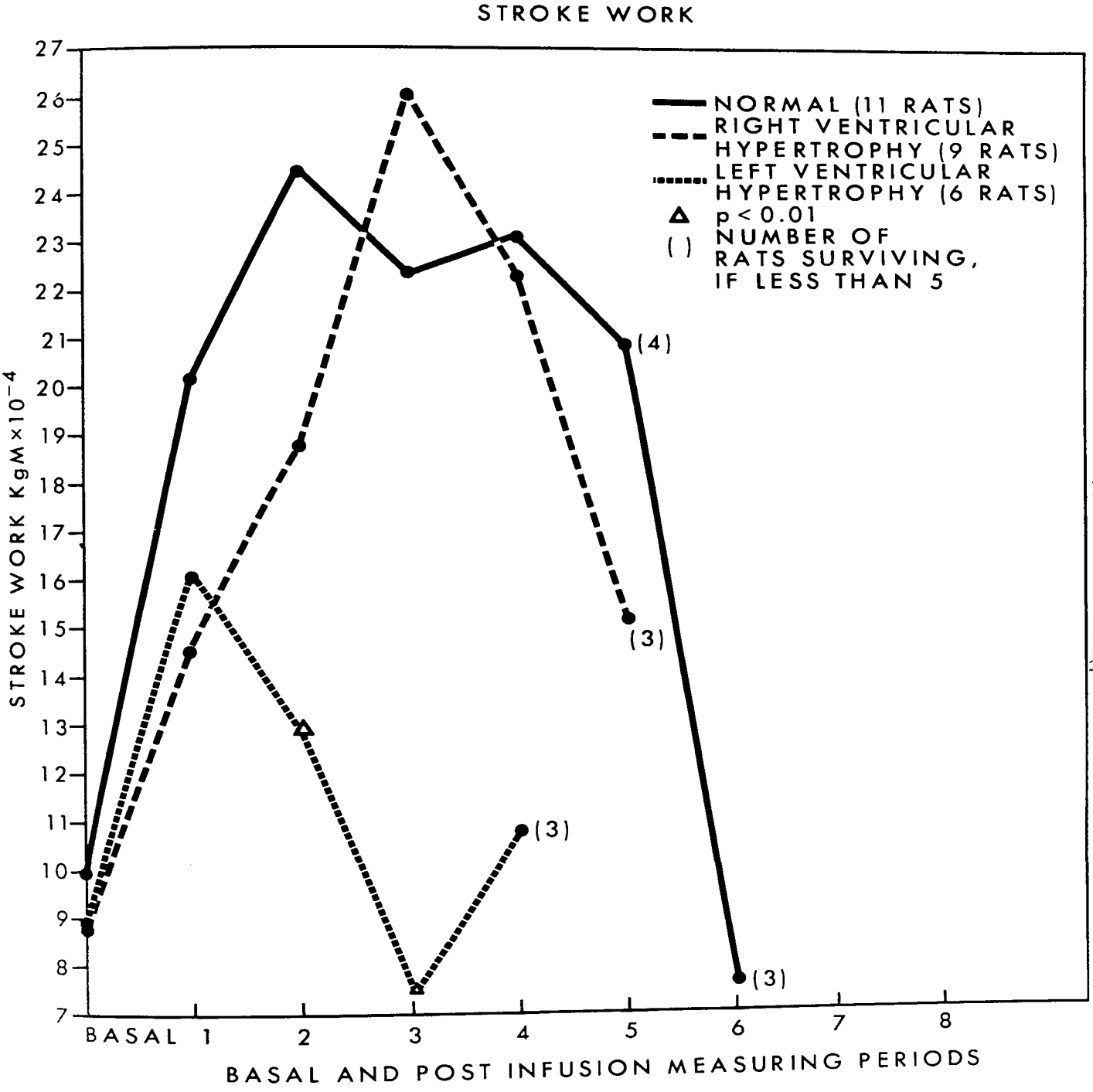


Figure 8

(5) Stroke Work

(a) Normal

The stroke work increased sharply to a peak value at PMP 2 and then slowly decreased.

(b) LVH

The stroke work increased to a peak at PMP 1 and then decreased sharply, the values at PMP 2 and 3 were both significantly below normal.

(c) RVH

The stroke work of the RVH group rose to a maximum value at PMP 3 and then fell away rapidly, none of these values differed significantly from normal.

(6) Developed Pressure. (Table 3)

(a)

Graph 5: The RVEDP in both ventricles increased during infusion unit one and then decreased consistently with each subsequent infusion unit.

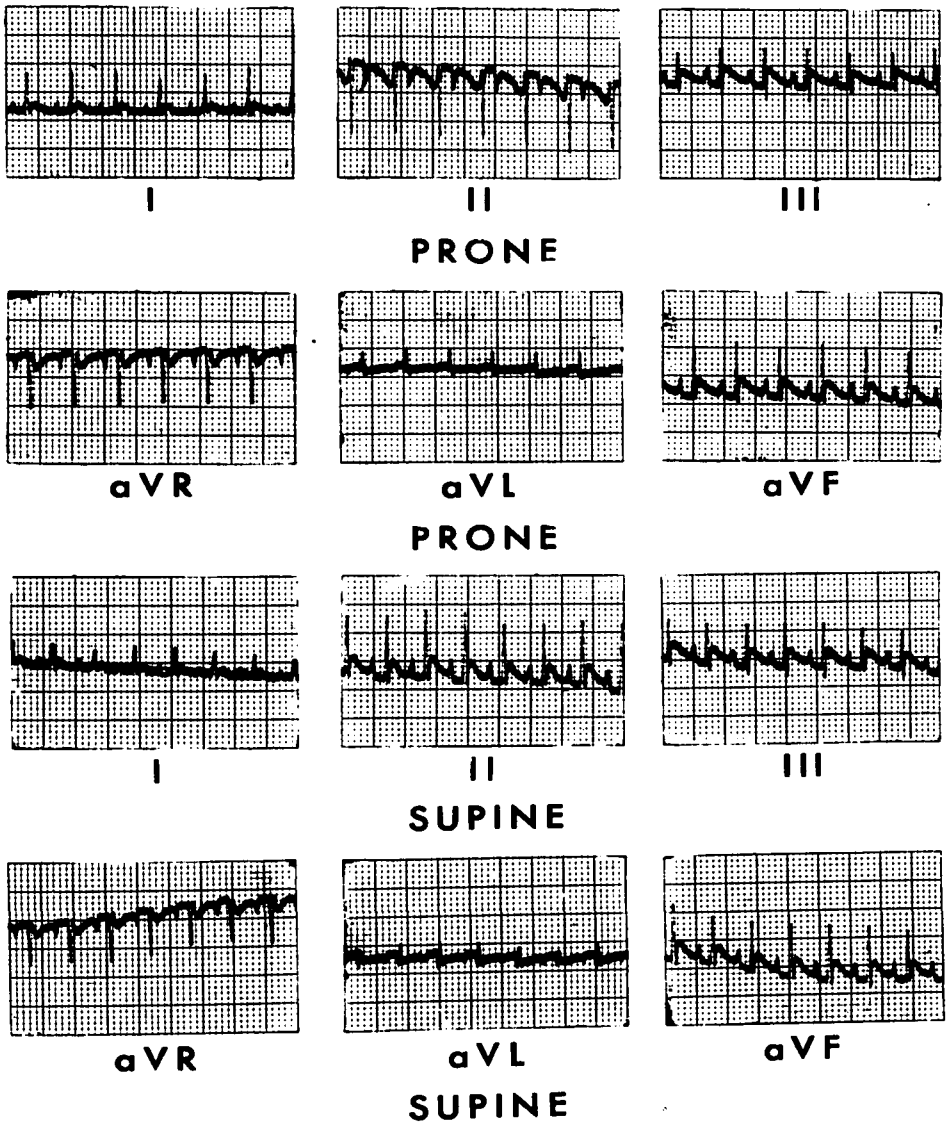
(b) Sham

The RVDP and LVDP did not differ significantly from that of the normal group, so were not included in this table.

(c) LVH

The LVDP increased to a very high value at PMP and then decreased markedly with each additional infusion unit; it remained above normal for the first 4 infusion units, being significantly so for the first 2.

The RVDP increased during the first infusion unit and remained at values above normal for the first 3 PMPs.



PAPER SPEED 50mm/sec CALIBRATION 1mv=20mm

1. ELECTROCARDIOGRAM (NORMAL RAT)

E. Preliminary Trials

(1) Electrocardiography

(a) Normal

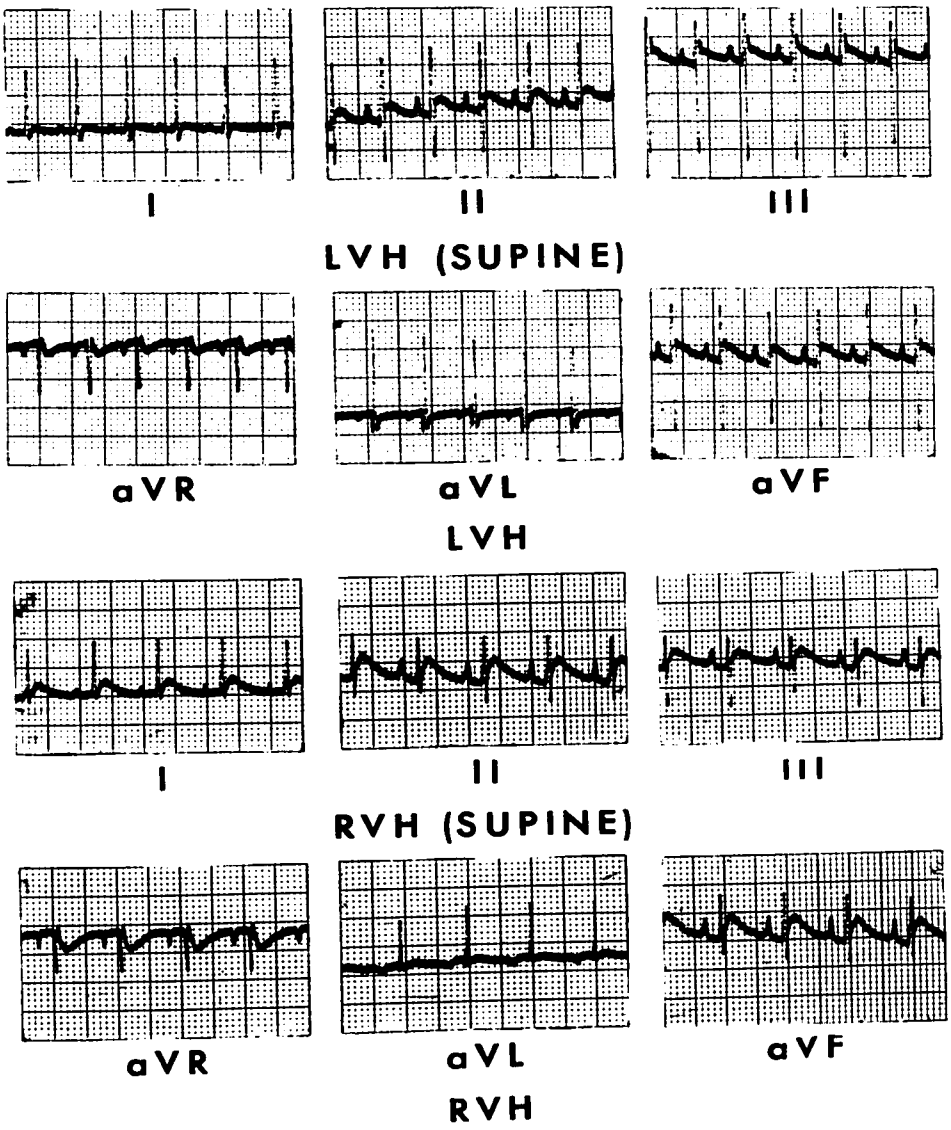
(i) P waves. The P waves were upright in leads 1,11,111 and AVF, inverted in AVR and tended to be flat in AVL. They were peaked and pointed in shape in all leads but AVL and were tallest in lead 11, indicating a P axis of approximately $+60^{\circ}$. The P wave deflections did not begin and end at a common isoelectric line but usually originated and terminated at different levels.

(ii) P-R segment. The P-R segments were usually sloping and often slightly wavy in outline, not following an isoelectric baseline. As neither the deflections nor the segments between them shared a common baseline the point at which the P-R segment ended and the QRS complex began was taken as the reference point for the isoelectric baseline for measuring the QRS complex.

(iii) QRS complex. Q waves were either absent or extremely rudimentary in all leads but AVR and AVL, in the latter they were usually small and rather distorted. The R waves were clearly discernible but the termination of the S waves were extremely difficult to distinguish as they blended into steeply rising T waves, without the interpolation of true ST segments. When calculating the QRS axis the S waves were assumed to terminate at the isoelectric line.

(iv) T and U waves. The T waves were asymmetrical in shape, the ascending limb rising steeply to a point, the descending limb being of gentler slope and longer duration. The descending limb of the T wave rarely returned to the isoelectric line before the origin of the P wave. Therefore true TP segments were not present.

U waves were not present.



PAPER SPEED 50mm/sec CALIBRATION 1mv=20mm

2. ELECTROCARDIOGRAMS (LVH AND RVH RATS)

(v) QRS Axis. The QRS axis of normal rats in the supine position was $+38.0^{\circ} \pm 8.0^{\circ}$ ranging from $+88.0^{\circ}$ to -2.0°. Nine of these animals had their ECGs recorded in the prone and supine positions. In the prone position the QRS axis was $28.0^{\circ} \pm 1.0^{\circ}$ and in the supine position was $35.0^{\circ} \pm 10.0^{\circ}$ - these results did not differ significantly from one another or from the preceding results. The amplitude of the QRS vector was 9.8 ± 1.1 mm for the 14 rats in the supine position.

(b) Sham

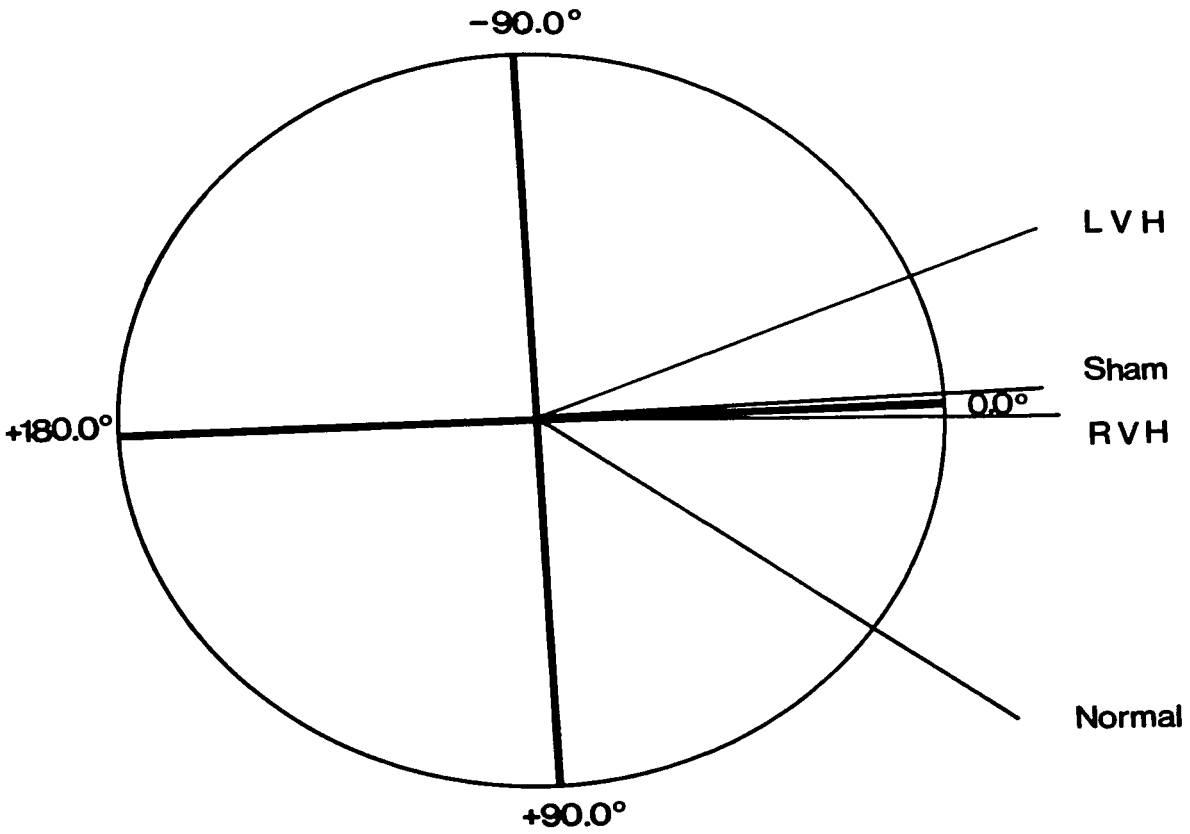
The electrocardiographic patterns of these rats did not differ from those of the normal rats.

In 7 sham operated rats the QRS axis in the supine position was $-0.5^{\circ} \pm 23.0^{\circ}$, this did not differ significantly from normal and in the prone position $-1.0^{\circ} \pm 17.0^{\circ}$, which did not differ significantly from that in the supine position. Three of these rats had QRS axes (-31.0°, -89.0° and -57.0°) which were far outside the range of normal values.

The amplitude of these axes did not differ significantly from those of the control group.

(c) LVH

The QRS axis of 12 rats in the supine position was $-20.5^{\circ} \pm 10.0^{\circ}$ (ranging from $+53.0^{\circ}$ to -37.0°) and was significantly different from the normal group but not the sham operated group. In 9 animals in which the ECG was recorded in both positions, the QRS axes ($-16.0^{\circ} \pm 8.0^{\circ}$ prone and $-25.5^{\circ} \pm 9.5^{\circ}$ supine) did not differ significantly from each other. The amplitudes of these QRS axes did not differ significantly from those of the normal group.



Normal : + 38.0° ± 8.0° (14 rats)

Sham : - 0.5° ± 23.0° (7 rats)

LVH : -20.5° ±10.0° (12 rats)

RVH : +2.0° ±16.0° (11 rats)

(N.B. rats in supine recording position)

3. QRS AXES IN FRONTAL PLANE

(d) RVH

The QRS axis of 11 animals from the RVH group, recorded in the supine position was $+2.0^{\circ} \pm 16.0^{\circ}$ (ranging from $+111.5^{\circ}$ to -40.5°) and was significantly different from the normal group but not from the sham operated group. The QRS axis recorded from animals in both the prone ($-6.0^{\circ} \pm 22.0^{\circ}$) and supine ($+2.0^{\circ} \pm 22.0^{\circ}$) positions did not differ significantly from normal. The amplitudes of these QRS axes did not differ significantly from those of the normal group.

(2) Contractility

As this was only intended as a preliminary trial, the number of animals in each group was not large enough to be analysed statistically for significance.

(a) Normal

In three normal rats the dP/dt/P was 172 sec^{-1} in the left ventricle and 240 sec^{-1} in the right ventricle, while the extrapolated V max (taking K=29 muscle lengths) was 5.9 muscle lengths sec^{-11} in the LV and 8.3 muscle lengths sec^{-1} in the RV.

(b) LVH

Only one rat with LVH was analysed and in this animal the dP/dt/P was 218 sec^{-1} in the LV and 153 sec^{-1} in the RV. The extrapolated V max was 7.5 muscle lengths sec^{-1} in the LV (27 % above the normal value) and 5.2 muscle lengths sec^{-1} in the RV (36 % below the normal value).

(c) RVH

In two RVH animals the average dP/dt/P was 145 sec^{-1} in the V and 112 sec^{-1} in the RV. The extrapolated V max was 5.0 muscle lengths sec^{-1} in the LV (15 % below normal) and 39 muscle lengths sec^{-1} in the RV (54 % below the normal value).

5. DISCUSSION

5. DISCUSSION

A. Production of Ventricular Hypertrophy

(1) LVH (Left Ventricular Hypertrophy

In agreement with the experience of other workers it was found that the margin between the degree of constriction which produced left ventricular hypertrophy and that which produced death from left ventricular failure was quite small. Ring 17 (internal diameter 1.40 mm, area 1.53 mm^2) produced a significant LVH with no mortalities, while Ring 18 (internal diameter 1.13 mm, area 1.02 mm^2) killed all animals. Nair (1968), working with 200-220 g rats, found an even narrower margin between these two extremes, bands smaller than 1.23 mm in diameter (area 1.20 mm^2) producing LV failure and death in all animals, while those of 1.34 mm in diameter (1.40 mm^2) produced a significant LVH with no deaths. Lipana and Fanburg (1970) examined the effects of 4 different ring sizes on the ascending aorta of rats weighing between 180 and 220 g, all animals with bands of 1.46 mm in diameter (area 1.68 mm^2) survived while those with bands of 1.068 mm in diameter died from heart failure. The extreme values found in this experiment and those reported by other workers are not sharp cutoff points for, as shown by Lipana and Fanburg, between them intermediary degrees of constriction are associated with intermediary mortality rates. Lipana and Fanburg found that bands of 1.27 mm in diameter were associated with mortality rates of 22 % and bands of 1.17 mm with a mortality rate of 50 %.

Once the size of the aortic ring had been chosen the mortality rate was only 16 % and was solely due to the surgical procedure. This was in the range designated by Beznak (1964) as acceptable (i.e. below 30 %) and similar to that reported by Stewart and Lindsay (1967) (18 %) when performing the same operation in smaller rats.

The weight gain of the LVH group during the period of constriction was below that of the sham operated group as well as the normal group, therefore decrease in the growth rate could not have been solely due to the effects of the thoracotomy. A decrease in the growth rate of rats with constriction of the ascending aorta, which could not be explained by the thoracotomy alone was also seen by Lipana and Fanburg (1970) after a constriction of one weeks duration and by Stewart and Lindsay after 9 weeks constriction.

The heart weight was always expressed relative to the body weight at catheterisation, as it has been shown that the changes in heart weight of the normal rat follow those of body weight very closely. Regardless of whether the rat has lost or gained weight the same cardiac weight always corresponds to a given body weight. (Walter and Addis 1939; Beznak 1954).

The heart weight, body weight ratio (HW/BW) changes as the rat ages; this relationship can be expressed by a regression equation, which will apply only to a specific strain of rat in a specific age group. Percentage changes in the heart weight of young rats should be calculated from the heart weights expected at specific body weights as determined by such an equation. However in this experiment, the changes in the HW/BW were calculated from a mean value obtained from 24 normal rats of the same strain and age group as those used to produce ventricular hypertrophy. The difference in mean body weights of the 4 different groups of rats used in this experiment was 50 g, a difference of approximately 10 % in body weight which, in the 400-500 g range in body weights, results in a difference of only 2 % in the HW/BW; so it was felt that no significant error would be introduced by determining the percentage changes in this ratio from a mean value. If, instead, the rats used in this experiment had weighed between 100 and 200 g, a 50 g difference in body weight would have resulted in a 12-14 % difference in the HW/BW and an unacceptable error would have been introduced, if the changes in the HW/BW had been calculated from a mean value. (Beznak 1954; Beznak 1967).

The percentage increases in left ventricular weight, (28.8 % and 36.7 % produced by Rings 16 and 17 respectively) were above 20% of the upper limits of the normal variation of the heart weight for a given body weight and could therefore be regarded as a true hypertrophy. (Korecky et al. 1966). The overall percentage increase in heart weight was naturally a little lower than the percentage increase in left ventricular weight but was within the range of 21 to 32 % reported by other workers in adult rats (of approximately 200 g) using rings of similar dimensions to those used in this experiment. (Hajdu and Beznak 1947 heart weight increases 32 % ring diameter 1.45 mm; Kerr et al. 1961 heart weight increases 30 % ring diameter 1.50 mm; Nair et al. 1968 heart weight increases approximately 30 % ring diameters 1.51 mm and 1.33 mm; Lipana and Fanburg 1970 heart weight increase 22 % ring diameter 1.46 mm). Greater percentage increases could be obtained in adult rats by increasing the degree of constriction but these were associated with unacceptable mortality rates, e.g. Lipana and Fanburg (1970) heart weight increase 50 %, ring diameter 1.17 mm and mortality rate 50 %.

The degree of hypertrophy produced by banding the ascending aorta of weanling rats was twice that produced in adult rats. Stewart and Lindsay (1967) used bands of 1 mm in diameter in 40 g rats, after 9 weeks of constriction the HW/BW had increased by 65 %, the LV free wall/BW by 63 %, the septum by 61 % and the RV free wall/BW by 35 %. However this increase was not as great as the 100 % increase in heart weight produced in rats fed an iron deficient diet for 100 days after weaning. (Korecky et al. 1964) In both of these experiments it is quite possible that this large increase in heart weight was due to hyperplasia (in the very young rat) as well as hypertrophy of the muscle fibres.

(2) RVH (Right Ventricular Hypertrophy)

The growth rate of rats with pulmonary artery rings was very poor indeed as they were still 10-20 g below their operative weights at the time of catheterisation. This is in agreement with the results obtained by Stewart and Lindsay 1967 who found that banding the pulmonary artery was associated with more severe stunting than banding the aorta.

Not surprisingly the LV was better able to cope with the strain of arterial constriction than the right ventricle. The right ventricle had to undergo a greater degree of hypertrophy when the diameter of the pulmonary artery was reduced by 32 % than the left ventricle found necessary to deal with a reduction of 45 % in the diameter of the aorta. At equal degrees of constriction (40 % decrease in diameter) the LVH group had developed an acceptable degree of hypertrophy but in the RVH Group the needs of the right ventricle had outstripped its blood supply and areas of fibrosis were present.

Stewart and Lindsay (1967) are the only other workers who have reported using pulmonary artery constriction to produce RVH in the rat. However their rats were much younger and the percentage increase in heart weight therefore much greater than this experiment. They applied rings of internal diameter 0.75 mm to the pulmonary arteries of weanling rats producing a cardiac hypertrophy of 69 % in 9 weeks. All parts of the heart had increased but the biggest increase was in the free wall of the right ventricle (free wall RV/BW 149 %; free wall LV/BW 38 % and septum/BW 14 %).

The use of banding to produce hypertrophy has several advantages: it requires only simple equipment and once the operative technique has been mastered it can be performed on a large number of rats at a time, the rats require no post-operative treatment and will develop an acceptable degree of hypertrophy very quickly. Other methods demand elaborate equipment

(such as the decompression chamber necessary to produce chronic hypoxic hypoxia) while others are very time consuming, demanding daily or regular attention to diet (e.g. anaemia), injections (e.g. cobalt, thyroxine) or supervision (e.g. exercise) for long periods of time. In arterial banding the stimulus is isolated and effects only the heart, not altering or damaging other tissues of the body; in addition, if the degree of constriction is carefully controlled it will only result in hypertrophy, and not in damage, of the cardiac tissues. Constriction of the ascending aorta, rather than the abdominal aorta was chosen, as the latter also results in ischemic changes in the lower portion of the body, particularly in the kidney. On the other hand the speed with which banding produces hypertrophy argues against its use when a more "physiological" hypertrophy is required; in this case a graded intervention is superior.

B. Cardiac Output

(1) Normal

After maturity the cardiac output of the rat per Kg BW, slowly decreases with age. As the rats used in this experiment were heavier and therefore older than any used in previously reported cardiac output determinations, it was not surprising that the cardiac output per Kg BW obtained in this experiment was lower than any of the already published values. Considering the cardiac output determinations made in normal rats, using Pentobarbital sodium anaesthesia, at dose rates similar to that used in this experiment, it can be seen that the cardiac output decreases from values of approximately 290 ml/min/Kg in 150 g rats, to 230-250 ml/min/Kg in 200 g rats and then to values in the low 200s in 250-300 g rats. (See Table 1). The cardiac output of the heaviest rats measured, using Pentobarbital sodium anaesthesia, was 204 $\pm$ 18 ml/min/Kg (in 305-345 g rats) (Popovic and Kent 1964) so the value obtained in this experiment, 196.3 $\pm$ 24.2 ml/min/Kg, in 425-525 g rats appears to be in agreement with those of other workers.

(2) LVH

The basal cardiac output of the normal group did not differ significantly from that of the LVH group. This result agrees with that obtained by Beznak (1958) and (1962) that the cardiac output of rats with LVH (produced by constriction of the abdominal aorta) is practically the same as that of normal rats of the same body weight.

(3) RVH

The only other workers to produce RVH by pulmonary artery constriction, in the rat, did not measure the cardiac output. The two other methods commonly used to produce RVH in the rat, chronic hypoxic hypoxia and polycythaemia, both alter the cardiac output per se by causing hypervolaemia (which tends to increase the cardiac output) and increasing the haematocrit (which tends to decrease the haematocrit output). No cardiac output determinations of the intact rat with RVH could be found but Souhrada (1967) used heart lung preparations to measure the cardiac output of rats with RVH produced by polycythaemia (resulting from cobalt injections) and found it to be elevated above normal.

C. Other Parameters

(1) Pressures

(a) Frequency Response

It was generally believed that instruments with a uniform dynamic sensitivity to the 10^{th} harmonic of the fundamental frequency were necessary to accurately measure physiological pressures. Since the heart rate of human beings rarely exceeds 240 beats per minute (fundamental frequency 4) an instrument with a uniform sensitivity from 0 to 40 cps would be required. However using an instrument with such a high frequency response introduced many artefacts, which were due to pressures generated within the catheter by accelerations and deccelerations of the fluid column, associated with movements

of the catheter usually imparted by the heart beat. (Wood 1954). More recent evidence indicated that manometer systems with a uniform frequency response from 0 to 10 cps would record the physiological pressures of man without significant amplitude distortion while eliminating high frequency artefacts, although low frequency artefacts of the same fundamental frequency as the heart beat can only be eliminated by a micromanometer situated at the tip of the catheter. Yarnof (1965) believes that physiological pressures can generally be recorded by using an instrument with a uniform sensitivity to the fifth harmonic, so that at a heart rate of 180 beats per minute (3 cps) a frequency response of 15 cps is required.

As the heart rate of rats is much higher than that of humans, an instrument used to record their physiological pressures must have a much higher frequency response. In this experiment the mean basal heart rate of the normal group was 385 beats per minute therefore, the fundamental frequency was 6.4 cps and the fifth harmonic 32.1 cps. The right ventricular catheter manometer system had a frequency response of 34 cps and was therefore adequate for recording the right ventricular pulse pressure of most normal rats. 34 cps corresponds to the fifth harmonic of a heart rate of 408 beats per minute. In all the basal Normal and RVH and LVH values only two rats had heart rates higher than 408 beats per minute so only in these two rats would amplitude distortion due to inadequate frequency response be expected. With commencement of the infusion the heart rates of all rats decreased so at PMP 1 and all PMPs thereafter the heart rates of all rats in each of the three groups were below 408 beats per minute and the frequency response of the right ventricular catheter manometer system could be considered adequate.

The frequency response of the LV catheter manometer system, 29 cps, was lower than that of the right as the LV catheter (internal diameter 0.02") was narrower than the RV catheter (internal diameter 0.03"). 29 cps corresponds to the fifth harmonic of a heart rate of 348 beats per minute. In the normal group the mean heart rate of the basal PMP, PMP 1 and PMP 2 were all higher than this value, (385 $\pm$ 4, 372 $\pm$ 6 and 357 $\pm$ 7 respectively) this was also true of the LVH group (basal 393 $\pm$ 7, PMP 1 389 $\pm$ 9 and PMP 2 358 $\pm$ 11) so amplitude distortion due to inadequate frequency response would be expected. However in the RVH group the heart rate was significantly lower than normal (basal 326 $\pm$ 14) so only in this group was the frequency response high enough to measure the LV pulse pressure accurately in the basal and all post infusion measuring periods.

To improve the frequency response of the left side catheter manometer system the internal diameter of the left ventricular catheter would have to be increased, but the extent to which this can be done, without interfering with cardiac function, is limited by the diameter of the aortic valve and the ascending aorta; technically it is limited by the even smaller diameter of the right carotid artery. The LV catheter obliterated approximately 14 % of the cross sectional area of the ascending aorta, leaving 86 % patent, from evidence gained by examining the cardiac outputs and LV pullouts this did not appear to hinder cardiac function. The RV catheter did not appear to block the venous return as the cardiac outputs were in the normal range, but if a catheter of this size were used to catheterise the LV 42 % of the cross sectional area of the aorta would be obliterated, leaving only 58 % patent. This degree of obstruction is similar to that created when LV catheters were passed through aortic rings (37 % of area of Ring 16 obliterated and 43 % of Ring 17) and was found to be detrimental to cardiac function.

Finally a catheter of these dimensions is too large to be advanced down the right carotid artery.

As the frequency response of the catheter manometer system used to record the physiological pressures of the rat had to be above 10 cps it recorded high, as well as low frequency artefacts. Artefacts were particularly obvious on the peaks of the LV pressure tracings from the normal rat. (See pressure tracing 1).

(b) Normal

The normal intraventricular pressures recorded by cardiac catheterisation in this experiment were considerably higher than those previously obtained by Radzievskii and Kapel'ko (1969) using ventricular puncture in the open chested rat, weighing 180-200 g, (LVSP 79.0 mm Hg, LVEDP 0.8 mm Hg, RVSP 23.2 mm Hg and RVEDP 2.8 mm Hg). In the open chested animal the venous return is reduced, it can no longer be aided by the respiratory movements, the size of the heart shrinks and the end-diastolic volume is drastically reduced. Most workers quote 110-120 mm Hg as the mean arterial pressure of rats in this weight range (e.g. Beznak 1954) and the LVSP must obviously be higher than these values. For these reasons it is felt that the cardiac pressures measured by catheterisation were more accurate than those measured by needle puncture in the open chested rat.

Lipana and Fanburg (1970) measured the LVSP of 180-200 g rats, by transdiaphragmatic puncture of the LV with a 21 or 22 gauge stainless steel needle inserted through an abdominal incision. They found the peak LVSP to be 126 $\pm$ 4 mm Hg in rats which had undergone a thoracotomy and a sham aortic constriction 7 days earlier. This result was obviously more accurate than that obtained by Radzievskii and Kapel'ko and may have been lower than that obtained in this experiment for three reasons. Firstly opening the abdomen would be expected to cause a moderate reduction in a venous return. Secondly as the internal diameter of

a 21 gauge needle (0.046 mm) is less than that of the LV catheter used in this experiment and a direct writing instrument was used to record the pressures, rather than a cathode ray oscilloscope and camera as in this experiment, the frequency response was probably poorer than that experienced in this experiment and may have resulted in a greater reduction in amplitude of the recordings. Thirdly, as already mentioned, most workers quote values of 100-120 mm Hg for the mean arterial pressure in 200 g rats, unfortunately Lipana and Fanburg did not give the mean arterial pressure of their rats but assuming that it was within this range the LVSP is only a little lower than would be expected. On the other hand the rats used in this experiment had a much higher mean arterial pressure (140.8 $\pm$ 3.9 mm Hg), which was partly because the rats were older and partly due to strain difference - other workers using these rats have consistently obtained higher values for mean arterial pressure than are generally regarded as normal - so, in turn, a much higher LVSP would be expected.

The LV pullouts confirmed that the LV catheter did not produce a relative stenosis of the aortic valve in normal rats as the peak systolic gradient across the valve did not exceed 20 mm Hg, the level indicative of mild aortic stenosis. As none of the ventricular pressure tracings showed any evidence of aortic or tricuspid regurgitation it was assumed that the catheterisation procedures had not injured the valves and that the catheters themselves did not cause an insufficiency of the valves.

The systolic arterial pressure is augmented as it moves towards the periphery - this is clearly shown in pressure tracing 1 - however the diastolic and mean arterial pressure decreases slightly.

(c) LVH

In accordance with the production of LVH the LVSP was increased above normal. Lipana and Fanburg (1970) also measured the LVSP after constriction of the ascending aorta - once again their results were lower than those obtained in this experiment. With an increase in the HW/BW of 21 % (they did not dissect the LV separately) they obtained an LVSP of 140 mm Hg; this degree of hypertrophy is comparable to that produced by Ring 16 in this experiment and in these animals the LVSP was 243 mm Hg. With increases in the HW/BW of 31 % and 42 % they obtained LVSPs of just below and just above 200 mm Hg respectively.

The LV pressure pulse exhibited the peaking typical of a dynamically significant degree of stenosis. The LV pullouts revealed pressure curves identical to those seen clinically in humans with the rare congenital lesion of Supravalvular Aortic Stenosis. In these patients the LVSP is high (up to 300 mm Hg) with a drop in systolic pressure in the proximal aorta. In the supravalvular chamber the pressure is arterial in contour, the systolic value is the same as that of the LV and the diastolic is the same as that of the aorta. (Zimmerman and Haghigi (1966).

The gradient across a stenotic valve or lesion is an indication of the severity of the lesion and is dependent on the area of the valve or region and the flow through it. For instance a critical aortic stenosis can exist with a relatively small gradient across the valve, if the left ventricular output is very low. If the cardiac output is normal (as in this group of rats) a gradient of 20-50 mm Hg suggests a mild stenosis, 50-100 mm Hg a moderate stenosis and 100-200 mm Hg a severe stenosis. (Kaplan 1966). Therefore in this group of rats normal basal cardiac outputs combined with very high gradients across the ring suggest a severe stenosis of the aorta.

Clinically, narrowing of the arterial pulse pressure is frequently, although not invariably, a finding in severe aortic stenosis. LV pullouts from LVH animals suggest that the LV catheter (which reduced the patent area of the ring by approximately 40%) contributed to the severity of the stenosis. In recordings, such as pressure tracings 3 and 4, the femoral artery pulse pressure is very damped but as soon as the catheter is pulled out of the ring the pressure pulse increases markedly.

(d) RVH

The RVSP exhibited the peaking typical of a dynamically significant degree of stenosis. The left side pressure curves did not differ from the normal group.

D. Changes During Infusion

(1) Survival Rates

In agreement with the results of Beznak (1959) most rats in the normal group survived 6 infusion units of PVP solution, but the survival rates were lower in the RVH group and lower still in the LVH group. Although these results do not indicate which side of the heart is better able to cope with a volume overload, they do show that hypertrophy of one ventricle decreases the heart's ability to do so and that hypertrophy of the LV is more deleterious in this regard than hypertrophy of the RV.

(2) Cardiac Output

Considering the cardiac output of the ventricles as a whole, both hypertrophy groups were able to maintain cardiac outputs and stroke volumes not significantly different from the normal for the first infusion unit, but after this the LVH group fell significantly below normal and remained there for the rest of the infusion. This latter result differs from that obtained by Beznak (1958) with another group of LVH rats; although the resting cardiac outputs of these rats did not differ from

normal, during the infusion the output was raised to values well above normal. Two factors may have contributed to this difference. Firstly Beznak produced LVH by constricting the abdominal aorta so the peripheral resistance above the ring was able to decrease, whereas in this experiment the ring was placed several mm from the aortic valves. Secondly the peripheral resistance in this experiment was further increased by the LV catheter, which passed through the aortic ring reducing the constricted area to approximately 60 % of its pre-catheterisation patency. As rats with LVH due to abdominal aortic constriction were able to increase their cardiac outputs significantly during volume loading it seems most likely that the inability of the rats in this experiment to do likewise was due to the very high and irreversible peripheral resistance rather than an intrinsic weakness of the heart due to hypertrophy of the left ventricle per se. (The output of the right ventricle was measured in this experiment but this equals that of the left ventricle until one of the ventricles fails.)

When considering the output of the ventricles as a whole, the RVH group seemed to do as well as the normal group but when considering it per gram RV tissue the values fell significantly below normal for the first two PMPs. This was not unexpected as it has been shown that a ventricle can increase its performance to or above normal by increasing its muscle mass but the performance of each muscle mass unit is reduced below normal. At PMP 3 the stroke volume per gram RV tissue rose, inexplicably, into the normal range. This increase could not have been due to anaemia as the haemoglobin did not fall below 7 g % (the value at which Beznak (1969) found that the cardiac output of the rat begins to increase due to anaemia) until PMP 5.

When heart failure occurs in hyperkinetic states (e.g. beri beri, hyperthyroidism) the cardiac output falls from its peak value but is still considerably above normal. In experimental volume overloading similar conditions prevail, peak values were seen in the normal group at PMP 4, in the LVH group at PMP 2 and in the RVH group at PMP 3, in this last group a second increase was seen during infusion unit 5 which was probably due to the haemoglobin falling below 7 g %. Similar results were obtained by Beznak (1959) in normal 200 g rats, peak cardiac output values were obtained at PMP 3, probably earlier than this experiment because the rats were lighter, and at PMP 2 in rats with a 45 % LVH (resulting from constriction of the abdominal aorta) and a second, but lower, elevation of the curve was seen at PMP 5. As cardiac outputs were not monitored continuously the actual peak values may have occurred in between the measuring periods and the values obtained at the measuring periods may represent points on the ascending or descending limbs of the true peaks.

(3) End-diastolic Pressure

(a) General

The Frank-Starling or ventricular function curve is the relationship between the mechanical activity of the ventricle (measured as stroke volume, stroke work etc.) and an index of end-diastolic fibre length (e.g. EDP, EDV). Electron microscopic studies have recently revealed the ultrastructural basis of this relationship. These studies have shown that the muscle fibres contain overlapping layers of contractile protein myofilaments, actin and myosin, which are arranged within a basic functional unit called the sarcomere. With increasing stretch (i.e. increasing fibre length) less over-lapping of the actin filaments occur at the centre of the sarcomere

and a greater number of contractile sites are available between the actin and myosin, leading to a quantitative increase of the force generating reactions and hence a higher point on the ascending limb of the ventricular function curve. The point of optimum sarcomere stretch is that at which there is maximum interaction between the actin and myosin; this provides the greatest degree of ventricular performance and corresponds to the apex of the ventricular function curve. When the sarcomeres are stretched beyond this optimum point the myofilaments become disengaged, less tension is produced and the ventricle operates along the plateau, and eventually along the descending limb, of the ventricular function curve.

The optimum sarcomere length is 2.2$\mu$and has been found to occur in the normal LV at an EDP of 10-12 mm Hg and at slightly less in the RV (Spotnitz et al. 1966; Leyton et al. 1970). In this experiment a rise in ventricular end-diastolic pressure above the values associated with optimum fibre length was interpreted as an indication of ventricular failure.

If the compliance (the relationship between the EDP and the EDV) is normal the EDP can be used as a good indication of EDV and hence end-diastolic fibre length, but if the compliance changes this is not true. In clinical cases of ventricular hypertrophy in the human the compliance is decreased and normal EDVs may be associated with excessive elevation of EDP. However a change in compliance implies a fundamental change in muscle property but studies of animals with experimental ventricular hypertrophy or failure have not found any evidence of abnormal diastolic compliance, either in the isolated muscle or in the intact ventricle (Williams et al. 1966, Spann et al. 1967). Why there is this difference between clinical and experimental hypertrophies is unknown.

In this experiment there was no difference between the basal and post-infusion RVEDPs of the two sub-groups (of the RVH group) and their degree of hypertrophy differed by 32 %, so any decrease in compliance could not have been significant in this case. From this result and the evidence found by other workers it is presumed that a decrease in compliance did not take place in the RV of the RVH group and in the LV of the LVH group.

In clinical cases of volume overload (due to polycythaemia etc.), in the absence of heart disease, the ventricles may move up the ventricular function curve but the resultant rise in EDP does not always indicate myocardial failure and should remain within the limits of 10-12 mm Hg, or less, in the absence of an abnormality in myocardial compliance or contractility. In severe cases of polycythaemia vera the blood volume may be twice normal. The blood volume of the rats used in this experiment would have been approximately 25 ml (Sjostrand 1962), this quantity of infusion was delivered by PMP 4, by which time the EDP had exceeded the 10-12 mm Hg in all ventricles. As the EDP rose above 12 mm Hg in each group, on each side of the heart before the blood volume had been doubled, it is assumed that these increases represent true ventricular failure and not merely artificial elevation due to the volume overloading.

(b) Left Side Pressures

The LVEDP of the LVH group rose rapidly passing the values associated with optimum sarcomere length towards the end of IU 1, while those of the RVH and Normal groups did not do so until the middle of IU 2. The LVEDP of the LVH group just exceeded 12 mm Hg at PMP 1 and rose rapidly to values indicative of ventricular failure during IU 2, by PMP 2 all groups had reached such values.

(c) Right Side Pressures

Again the hypertrophied ventricle showed itself less able to cope with the strain of volume overloading, the RVEDP of the RVH group rose past the values associated with optimum sarcomere length in IU 3, while those of the normal and LVH group did not do so until IU 4.

The mechanical activity of the right ventricle, as measured by its stroke volume, or cardiac output determined by the direct Fick method, correlated fairly well with the changes in RVEDP. As the RVEDP rose the RV stroke volume increased along the ascending limb of the ventricular function curve. Near values associated with optimum sarcomere length peak stroke volumes were seen in the RVH and normal groups and then, as EDP increased even further, the stroke volumes began to fall. Optimum sarcomere length was probably achieved during IU 3 in the RVH group and IU 4 in the normal group, accordingly peak stroke volume and cardiac output values were seen at PMPs 3 and 4 respectively. As the cardiac output was not monitored continuously these were probably not actual peak values but were more likely to be points near the peak values, on the descending limb of the cardiac output curve. However in the LVH group the stroke volume peaked at PMP 2, when the EDP was between 5 and 6 mm Hg; possible reasons for this will be discussed later.

(d) Comparison of Right and Left Side Pressure

Assuming that neither changes in compliance nor volume loading have artificially elevated the ventricular end-diastolic pressures it can be concluded that the LV fails before the RV in normal rats and those with right or left ventricular hypertrophy during volume overloading.

(4) Systolic Pressures

(a) Left Side Pressures

The LVSP of all groups rose to peak values at PMP 1. Optimum sarcomere length was probably reached towards the end of IU 1 in the LVH group and during IU 2 in the normal and RVH groups, so the values measured at PMP 1 probably represent a point on the descending limb of the LVSP curve of the LVH group and points on the ascending limbs of the normal and RVH curves. At PMP 2 the LVSP of all groups had decreased, this was in accordance with the findings that the LVEDPs of all groupds had passed the values generally associated with optimum sarcomere length. These changes in LVSP naturally influenced the mean femoral artery pressure, which exhibited a similar rise during the first infusion unit and a continuous fall thereafter. Identical changes in blood pressure were seen by Beznak (1968) when performing the same infusion experiment on 200 g rats.

The LVSP of the RVH group was consistently below that of the normal group and was clearly the lowest trace on graph 7. This is an interesting finding as in cases of Cor Pulmonale, in which there is an RVH resulting from an increased work load on the right ventricle due to alterations in the pulmonary circulation, the performance of the LV is often found to be decreased an in chronic cases LVH and failure may be present. The reasons for this are not known but various suggestions include impairment and overstretching of anatomically continuous muscle layers, elevation of right atrial pressure decreasing the perfusion gradient across the coronary bed, thereby diminishing oxygen delivery to the LV and, in chronic cases, increased flow in the bronchopulmonary vascular shunts, increasing the work and output of the LV.

(b) Right Side Pressures

The RVSP of the LVH group reached its peak value at PMP 2 which coincided with the peaking of the right ventricular cardiac output, but not with the period of optimum sarcomere stretch. The RVSP of the RVH group was sustained at extremely high values for PMPs 1, 2 and 3 but had decreased by PMP 4, this decrease was associated with a simultaneous fall of the right ventricular cardiac output from its peak value and an increase in the RVEDP above the values representing optimum sarcomere stretch. The RVSP of the Normal group decreased below the basal value as the cardiac output fell and the RVEDP rose above the normal limits.

(5) Developed Pressure

The changes in developed pressure mirrored those seen in systolic pressure.

(6) Cardiac Work

Cardiac work as determined in this experiment was composed of values from both sides of the heart, i.e. cardiac output of the right ventricle and systemic arterial blood pressure, as such it was incapable of differentiating between the performances of the ventricles. The cardiac outputs of the ventricles are equal until one fails, so cardiac work calculated in this manner is an accurate indication of left ventricular work until one or other of the ventricles fail. The LV of the LVH group had to perform extra work overcoming the resistance of the stenotic region so the mean pressure in the supravalvular chamber was used instead of that in the femoral artery, to calculate cardiac work. The mean pressure in the supravalvular chamber is represented by the mean of the LVSP and the diastolic pressure in the ascending aorta; as the ascending aorta was not catheterised during the infusion experiments the diastolic pressure in the femoral artery was used instead, this may have been a few mm Hg lower than that in the aorta but would not have introduced a significant error.

In the LVH group maximum cardiac work occurred at PMP 1, coinciding with the peaking of the LVSP, before failure of the LV, so could be taken as representative of the true work of the left ventricle. In the normal and RVH groups maximum cardiac work occurred after failure of the LV, at PMPs 2 and 3 respectively, coinciding with the maximum cardiac output values in the RVH but not the normal group. If the left ventricular output had decreased significantly below that of the right after left ventricular failure the rats would have rapidly died from acute pulmonary congestion but, as they continued to live for 30 to 40 minutes, the cardiac output of the two ventricles could not have been too dissimilar and the cardiac work, calculated in this manner, must still have been a good approximation of the left ventricular work.

(7) Comparison of the Changes Seen in All Parameters During Infusion

From the changes in EDP it was concluded that the left side of the heart of normal rats and those with RVH or LVH failed first during volume overloading - this conclusion was also supported by the changes which were seen in the cardiac output and the intraventricular systolic pressures. In all groups the LVSP reached peak values when the LVEDP was at values associated with optimum sarcomere length and then proceeded to fall as the EDP increased to values generally associated with ventricular failure. After failure of the LV the RVEDP, RVCO and RVSP continued to rise, when the EDP of the normal and RVH groups reached values typical of ventricular failure the RVSP and the RVCO fell. In the LVH group slightly different conditions prevailed, as the RVCO and RVSP commenced to fall while the RVEDP was still within the normal range.

The left ventricular failure which occurred in this experiment corresponded to that seen in high output failure in which the heart is no longer capable of working at maximum performance but the cardiac output

is maintained at a high level. That the cardiac output of the two ventricles could not have differed greatly after failure of the left has already been discussed, so it can be seen that the LVCO of the normal and RVH groups continued to increase until the RV and hence the whole heart failed. However the animals with aortic rings were not able to increase their LV cardiac outputs in a similar manner, presumably due to the irreducible increase in peripheral resistance caused by the aortic ring and catheter, thereby reducing the venous return to the right ventricle and hence the RVCO and RVSP decreased despite the fact that the RVEDP had not yet reached failure levels.

It was concluded that during experimental volume overloading, in normal rats and those with RVH or LVH, the left ventricle fails before the right, so its failure is probably not influenced by that of the right ventricle. Therefore the maximum cardiac work, measured under these conditions is a valid index for comparing the LV performance of various groups of rats.

E. Preliminary Trials

(1) Electrocardiography

(a) Frequency Response

The frequency response of the direct writing electrocardiograph used in this experiment (3db down at 100 cps) was not ideal for recording the ECG of the rat. Using cathode-ray oscilloscope techniques Angelakos and Bernardini (1963) performed a comprehensive Fourier Analysis of the ECG of the rat and found that the QRS complexes were composed of frequencies ranging from 50 to 400 cps. Goodwin and Fraser (1965) found that simultaneous ECG recordings made with a cathode-ray oscilloscope and a direct writing instrument (frequency response 3db down at 175 cps) were very similar and concluded that the "frequencies which influence the main pattern of the rat ECG are those below 200 cps." No appreciable difference in the main

characteristics of the ECGs could be detected in this study when comparing recordings made by the direct writing instrument (Appendix A8) and the cathode-ray oscilloscope and camera (Appendix A15), so it was concluded that no significant error was introduced by relying mainly on recordings made on the former.

However if a more detailed survey was undertaken it would be advisable to use an electrocardiograph with a cathode-ray oscilloscope and camera to ensure that the results would not be effected by the frequency response of the recording system.

(b) Distinctive Features of the ECG of the Rat

As this study was only intended as a preliminary trial the ECGs were not examined in great detail but the main features were noted and the QRS axis measured. The distinctive features found in this study, such as short pointed P waves, undulating sloping PR segment, lack of an isoelectric baseline and absence of ST and PR TP segments have also been reported by other workers and the characteristics of the P wave have been related to the atrial action potential (Beinfield and Lehr 1968a) but no report could be found relating the QRS complex and the T wave to the ventricular action potential.

Beinfield and Lehr (1968a) also reported very pointed P waves, of short duration, and a P axis of $+60^{\circ}$ in the ECG of normal rats. In addition they found prominent TP waves of variable configuration in the PR segment; the undulations seen on the recordings made with the cathode-ray oscilloscope in this study probably correspond to TP waves. Beinfield and Lehr suggest that the shape of the P waves and the appearance of TP waves was probably related to the very brief activation and recovery phase of the rat atria as well as the high heart rate and relatively long PR segment.

Previous workers differ in their interpretation of the ST junction in the ECG of the normal rat. Waller and Charipper (1945) and Kenedi (1968) believed that an ST segment was present while Sambi and White (1960), Beinfield and Lehr (1968b) Normann et al. (1961) and Werth and Wink (1967) thought that the S and T waves were fused without interpolation of an ST segment - which was also the interpretation of this study.

The ST segment is seen in the ECG of humans and other species when the whole of the ventricular myocardium is depolarised and corresponds to the period of slow repolarisation, or plateau, of the action potential of the ventricular muscle. (Burton 1965). The action potential of the papillary muscle of the rat has a rapid repolarisation phase and does not exhibit a plateau. (Lee et al. 1970). Although there are no reports of similar studies of the action potential in other areas of the rat myocardium it might be surmised that the lack of the ST segment in the ECG of the rat is due to the extremely rapid repolarisation of the muscle. This would also tend to confirm Beinfield and Lehr's suggestion that some points of the ventricle may be repolarising while some points are still depolarising.

The asymmetrical T waves, with long slow descending limbs, which were seen in this study were also reported by Sambi and White (1960) and Beinfield and Lehr (1969b) but Kenedi (1968) considered this deflection to consist of a T wave and a U wave - the latter being of multiform appearance. No U waves were detected in the recordings made in this study. The U waves seen in the human ECG are believed to represent repolarisation of the papillary muscle. Lee et al. (1970) report that the first phase of rapid repolarisation in the rat papillary muscle is followed by a phase of very slow repolarisation (similar to the after-discharge seen in skeletal muscle) and this may correspond

to the U waves seen by Kenedi or, as interpreted by others, the slowly descending limb of the T wave.

(c) QRS Axis

(i) Normal

The mean QRS axis is influenced by a number of factors, such as the position of the heart within the thorax, relative preponderance of right and left ventricles, rotation of the ventricles around the longitudinal axis, thickness of the ventricular walls and rate and sequence of ventricular conduction. When examining the QRS axis of a normal rat it is necessary to take into account age (usually reflected by the body weight) and position of the rat's body.

The proportion of right to left ventricle in the very young rat is much larger than in the adult rat but with increasing age the relative size of the left ventricle increases, resulting in a slow movement of the QRS axis towards the left. This was demonstrated by the results of Waller and Charipper (1945) who studied the ECGs of rats ranging in weight from 100 to 350 g, they found a preponderance of right axis deviation in the lower weight groups (100-150 g; 150-200 g), which finally disappeared in the highest weight group (300-350 g). Values of the mean QRS axis of normal rats previously published were all determined on lighter rats and were correspondingly further to the right than those in this study. Normal values include $+47^{\circ} \pm 33^{\circ}$ (SD) in 208 g rats (Fraser et al. 1967), $+52.0^{\circ} \pm 6.0^{\circ}$ (SE) in 254 g rats (Normann et al. 1961) and $+49.6^{\circ} \pm 22.6^{\circ}$ (SD) in 284 g rats (Beinfield and Lehr 1968b) as compared to $38.0^{\circ} \pm 10.0^{\circ}$ (SE) in 450 g rats in this study.

In this study the QRS axis differed according to whether it was recorded with the rat in the prone or supine position. Similar changes in the QRS axis with positional changes have been reported by Goodwin and Fraser (1965) and Beinfield and Lehr (1956) the latter workers recommended the prone recording position as it resulted in the most stable and consistent tracings. As the prone position corresponds to the normal anatomical position of the rat it would seem more sensible to use this recording position.

(ii) Sham

Although the QRS axis of the sham operated rats did not differ significantly from normal the axes of 3 out of the 7 animals exhibited severe left axis deviation. This suggests that in these three rats the operation may have altered the anatomical position of the heart - perhaps due to fibrous adhesions between the heart and the incision site in the left lateral wall. Although the electrical axis does not correspond to the anatomical axis of the heart it is altered by changes in orientation of the heart within the thorax, as seen with changes in body positions. From these results it appears that it is unwise to use rats with cardiomegaly produced by constriction of the ascending aorta or pulmonary artery to study the electrocardiographic changes taking place during the development of ventricular hypertrophy, and it would seem that it would be preferable to use animals with cardiomegaly produced by methods which do not involve thoracic surgery.

(iii) LVH

The rats in this group exhibited a moderate left axis deviation (i.e. QRS axis within the range of 0°-30°). Their QRS axis was significantly different from the sham operated as well as the normal group and so could be interpreted as an indication of moderate LVH.

(iv) RVH

The QRS axis of the RVH group was significantly different from the normal but not the sham operated group which suggests that the

apparent left axis deviation was only due to the thoracotomy. However no signs of right axis deviation were found, which was an unexpected result. If the thoracotomy had tended to move the axis to the left and hypertrophy of the right ventricle had tended to move it to the right, the resultant axis might feasibly be found in the normal range but not significantly to the left of normal. In addition a larger number of individual rats would have been expected to escape the effects of the thoracotomy and display right axis deviation.

This result indicates that changes in the QRS axis cannot be used to diagnose right ventricular hypertrophy in the rat and casts doubt on its usefulness in diagnosing left ventricular hypertrophy.

(2) Contractility

The contractility of both ventricles was successfully measured in 6 rats, using only a catheter manometer system. The results obtained on the left side were acceptable but cannot be regarded as accurate as those obtained on the right side for, as already discussed, amplitude distortion, due to inadequate frequency response, would be expected on the left side. Both high and low frequency artefacts were recorded with this catheter manometer system and may have effected the accuracy of these determinations. High frequency artefacts are unavoidable when recording intracardiac pressures in the rat, as the frequency response of the catheter manometer system must be over 10 cps, but low frequency artefacts could be eliminated by using a catheter tip manometer - if one small enough for use in the rat could be manufactured.

As this was only intended as a preliminary trial, the number of rats in each group was not large enough to be analysed statistically (the 6 rats used in the determinations consisted of 3 Normals, 1 LVH and 2 RVH rats).

In ventricular hypertrophy secondary to chronic pressure overload of the ventricle (in humans and experimental animals) the contractility (indicated by V max) has been found to be depressed below normal. (Mason et al. 1970). In this experiment the V max of the RV in the RVH group was below normal but, unexpectedly, that of the LV in the LVH group was above normal - no particular significance can be attached to these results as so few animals were analysed.

More work is needed to find a truly accurate index of contractility, which can be measured by only a catheter manometer system. Normal rats and those with RVH or LVH could be used in these studies but the results would be more accurate if it were possible to use a catheter tip manometer.

6. SUMMARY

SUMMARY

The work described in this thesis was undertaken to try and determine the validity of the assumption underlying the experiment which was designed by Dr. Beznak to evaluate the performance of the left ventricle (LV). In this experiment the indices of cardiac work and cardiac output rose to maximum values during the infusion of a solution of PVP (polyvinyl-pyrrolidone), these values were considered to be measurements of the reserve force of the LV. The assumption underlying this experiment was that the left ventricle would fail before the right, during volume overloading, and therefore it was the maximum performance of the LV which was being assessed, (uneffected by right ventricular failure). If this assumption was not valid, and the right ventricle failed first, it would have been the left ventricle's ability to cope with the volume overloading, while handicapped by a failed, and therefore inefficient, right ventricle, which was being evaluated. In this thesis this assumption was examined in normal rats as well as those with right ventricular hypertrophy (RVH) or left ventricular hypertrophy (LVH).

Initially methods for producing RVH and LVH, utilizing constriction of the pulmonary artery and ascending aorta respectively, were developed. Using these methods an adequate degree of hypertrophy was successfully produced in a large number of rats.

To enable comparison of the performances of the left and right ventricles during the volume loading experiment, intraventricular pressures had to be recorded. Intravascular catheterisation was chosen as the most accurate means of recording these pressures but, as no reference to the use of this procedure in the rat could be found, new techniques had to be developed. The techniques which were evolved entailed the manipulation of silastic catheters down the right external jugular vein and the right carotid artery to the right and left ventricle respectively. Clear

intraventricular pressure tracings were obtained from both ventricles. There is the possibility that there was some amplitude distortion in those obtained from the left side; this could have been avoided by increasing the diameter of the catheter but was not possible as it would have made the catheter too large to advance down the carotid artery and would have created a relative stenosis of the aortic valve.

Rats with left or right ventricular hypertrophy were catheterised as well as normal ones. In rats with LVH the LV catheter had to pass through the aortic ring, this was found to create a relative stenosis in itself. If similar experiments were to be performed in future it would be advisable to place the constricting ring on the aorta distal to the right carotid artery, so the LV catheter would not need to pass through it.

In all rats the basal cardiac output (determined by the direct Fick Principle), the femoral artery pressure, the right and left end-diastolic pressures and right and left peak systolic pressures were measured and from these the parameters of cardiac work and developed pressure were calculated. These measurements were then repeated at regular intervals after infusion of a set amount of PVP solution. The plan of the infusion experiment was the same as that designed by Dr. Beznak. From changes in end-diastolic pressure (EDP), supported by those in cardiac output and systolic pressure, the times of right and left ventricular failure were determined.

From changes in EDP it was concluded that the left side of the heart of normal rats and those with RVH or LVH failed first during volume overloading with PVP. The ventricle was said to have failed when the EDP rose above the pressures commonly associated with optimum sarcomere length in that ventricle. Use of this criteria, in this experiment, was based upon two assumptions:

1. That the compliance of the hypertrophied ventricles remained unchanged.
2. That the increase represented true ventricular failure and not merely artificial elevation due to volume overloading.

It is thought that these assumptions are valid for the following reasons:

1. Other studies of animals with experimental ventricular hypertrophy have not found any evidence of abnormal compliance and in this experiment there was no significant difference in the EDPs of the two sub-groups (of the RVH group) although their degree of hypertrophy differed by 32 %.
2. In clinical cases of volume overloading the blood volume may be twice normal and the EDP may rise as the ventricle moves up its ventricular function curve but, in the absence of heart disease, it will remain within the normal limits. In this experiment each side of the heart had failed before the blood volume had doubled, so it was assumed that this represented true failure and not merely an artifical elevation due to volume overloading.

In all groups the LVSP (left ventricular systolic pressure) reached maximum values when the LVEDP was at values associated with optimum sarcomere length and then proceeded to fall as the LVEDP increased to values generally associated with ventricular failure. The left ventricular failure which occurred in this experiment corresponded to that seen clinically in cases of high output failure in which the heart is no longer capable of working at maximum performance but the cardiac output is maintained at a high level. If the LVCO (left ventricular cardiac output) had decreased markedly below that of the right ventricle, the rats would have died rapidly of acute pulmonary congestion but instead they continued to live for 30-40 minutes, so the LVCO must have still been at a high, although not maximal, level.

After LV failure the RVCO (measured in this experiment by the direct Fick Principle) continued to rise, as the output of the two ventricles could not have been too dissimilar the LVCO must also have continued to rise, despite ventricular failure.

After failure of the LV the RVEDP, RVCO and RVSP continued to rise as the PVP infusion proceeded. When the RVEDP of the normal and RVH groups reached values typical of ventricular failure, the RVCO and RVSP fell, but in the LVH group these parameters started to fall while the RVEDP was still within the normal range.

As already mentioned, the output of the left and right ventricles could not have differed greatly after LV failure, so it can be seen that the LVCOs of the normal and RVH groups continued to increase until the RV, and hence the whole heart, failed. However this was not true for the LVH group. As another group of rats, with LVH due to constriction of the abdominal aorta, was capable of increasing its cardiac output under these conditions it was assumed that the inability of the LVH group in this experiment to do the same was due to the irreducible increase in peripheral resistance, caused by the ring on the ascending aorta and the LV catheter passing through it. The increased peripheral resistance reduced the venous return to the right ventricle and hence the RVCO and RVSP decreased, despite the fact that the RVEDP had not yet reached failure levels.

Cardiac work was calculated, as in Dr. Beznak's original experiment, as the product of the RVCO and the mean arterial pressure. The outputs of the right and left ventricles until one ventricle fails but, as already shown, even after failure of the LV in this experiment the output of the two ventricles could not have been very dissimilar and therefore cardiac work, calculated in this manner, must have been a good approximation of left ventricular work, both before and after LV failure.

In conclusion it has been shown that the assumption that the LV fails before the RV, during the volume loading experiment designed by Dr. Beznak, is valid. Therefore cardiac output and cardiac work, as determined in this experiment, are good indicators of the left ventricular performance during volume loading, both before and after LV failure, until failure of the RV occurs.

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R E S U M E

For a number of years Dr. Beznak and her colleagues in this laboratory have been evaluating the performance of the LV of rats under various different conditions. One of the major methods used to assess the performance of the LV left ventricle was its response to volume overloading with a solution of PVP (polyvinylpyrrolidone). During the infusion the indices of cardiac output and cardiac work rose to maximum values, which were considered to be a measure of the reserve force of the heart.

As the right ventricle copes more easily with a volume overload than the left, it was assumed that the left ventricle would fail before the right during this experiment and that it was the maximum work of the left ventricle, unaffected by right ventricular failure, which was being measured. As this assumption had never been proven, under these specific conditions, the experiments which are described in this thesis were undertaken to try and determine whether it was valid. This assumption was examined in normal rats as well as those with right or left ventricular hypertrophy.

The many different methods which can be used to create cardiomegaly in rats are discussed briefly in the Literary Review. From these, constriction of the pulmonary artery and ascending aorta were chosen to produce right and left ventricular hypertrophy respectively.

The experiment used to evaluate the cardiac performance of the group of rats was based on the one originally designed by Dr. Beznak. Basal cardiac output and pressure measurements were made with the animal anaesthetised and then repeated after the infusion of set volumes of a PVP solution. The cardiac output was measured by the direct Fick Principle, this and other methods which have been used to determine the cardiac output of the rat are described in the Literary Review, along with the results of previous determinations made by other workers.

Cardiac work was then calculated as the product of the cardiac output and femoral artery pressure. Although this parameter could be used as an index of left ventricular performance, it was not capable of differentiating between the performance of the right and left ventricles, so additional parameters were sought.

The problem of evaluating cardiac performance is a difficult one and is described in general terms, with particular emphasis on the performance of the heart as a pump, in the Literary Review. As many of the parameters of cardiac performance can only be obtained by cardiac catheterisation, techniques for catheterising the right and left ventricles of the rat were developed. This enabled simultaneous comparisons of right and left ventricular end-diastolic pressures, systolic pressures and developed pressures; the changes in these values, as well as those in cardiac output and cardiac work, made possible the accurate assessment of the point of failure of each ventricle during volume overloading.

Right and left ventricular hypertrophy was successfully produced in a large number of rats, using the surgical techniques which were evolved for this experiment. As no reference to intravascular cardiac catheterisation in the rat could be found, new techniques had to be developed; these involved the manipulation of silastic catheters down the right external jugular vein and carotid artery to the right and left ventricle respectively. Using these techniques clear pressure tracings were recorded from both ventricles of all rats. As this was the first accurate recording of the intraventricular pressures of the rat, some pressure tracings are included in the thesis.

From the changes in end-diastolic pressure, supported by those in cardiac output and intraventricular systolic pressure, it was deduced that the left side of the hearts of normal rats and those with left or right ventricular hypertrophy failed first during volume loading.

The ventricle was said to have failed when the end-diastolic pressure rose above the values commonly associated with optimum sarcomere length in that ventricle; this was accompanied with a fall in cardiac output and systolic pressure.

It was concluded that the assumption that the left ventricle fails before the right ventricle during the volume loading experiment designed by Dr. Beznak is valid.

After the main experiment two preliminary trials were undertaken. In the first the feasibility of using rats with right and left ventricular hypertrophy (produced by the methods developed in this experiment) to study the electrocardiographic changes taking place during the development of ventricular hypertrophy was investigated. In the second, the possibility of measuring the myocardial contractility of these rats, using only a catheter tranducer system, was explored.