HAMLIN, Robert Louis, 1933–
EVALUATION OF THE SIMULTANEOUS INDICATOR-
DILUTION METHOD FOR DETECTING MITRAL
REGURGITATION IN DOGS.

The Ohio State University, Ph.D., 1962
Physiology

University Microfilms, Inc., Ann Arbor, Michigan
EVALUATION OF THE SIMULTANEOUS INDICATOR-DILUTION METHOD
FOR DETECTING MITRAL REGURGITATION IN DOGS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By


*****

The Ohio State University
1962

Approved by

[Signature]
Adviser
Department of Veterinary
Physiology and Pharmacology
DEDICATION

My accomplishments—no matter how meager they may be—are related only casually to my few qualifications, and, instead, result from the good fortune of having studied and worked under two outstanding individuals. If I am to receive full credit for any facet of my education, it is for having the sagacity to seek these two men as preceptors. It is for this reason that I respectfully dedicate this dissertation to Dr. Herman K. Hellerstein, Professor of Medicine, Western Reserve University and to Dr. C. Roger Smith, Professor of Veterinary Physiology and Pharmacology, The Ohio State University.
ACKNOWLEDGMENTS

During my tenure as graduate student and researcher at The Ohio State University, I have been impressed greatly by the dependency of an individual upon his professors, counselors, laboratory assistants, and sources for financial support. His immediate family and friends need necessarily be included with this group, since their understanding must be solicited during the periods of preparation for examinations and other events which may stress even the most euphoric individual to misanthropy. I express my indebtedness to the following individuals and organizations, and acknowledge that, without them, my graduate education would have been impossible: my parents, Walter E. and Sara E. Hamlin, for providing me with an environment conducive to achievement; my brothers, Leon M. and Melvin L. Hamlin for providing me with images to which I could aspire; my wife, Beverly G. Hamlin, most helpful and understanding as only a wife can be; my uncle, Michael J. Gallin, who was responsible for my affection toward animals; Dr. Richard D. Lercey, my first contact with the veterinary profession, a true friend and one of the finest veterinary practitioners in the country; Drs. Richard W. Redding and C. Roger Smith,
preceptors in my pursuit of a graduate education, friends and outstanding contributors to the field of veterinary physiology and pharmacology; Dr. Herman K. Hellerstein, the source for my knowledge of cardiology and an individual with unique drive and accomplishments; Walter Krill, Dean of the College of Veterinary Medicine, The Ohio State University; Mrs. Myra Dull, for her patience; Dr. Leo A. Sapirstein, a friend and adviser in my research pursuits; Mr. William P. Marsland and Mr. Stephen Boggs, and Dr. David Smetzer for invaluable cooperation and assistance in my research; Dr. Allen M. Scher, Professor of Physiology at The University of Washington; the Central Ohio Heart Association, in particular Miss Jeanne James; the National Institutes of Health, for financial and moral support of my research interests; and the Martin Company, in particular Dr. Harry Gorman, for the support of one important phase of my research activity.

To all of the above, and, I am certain, to many others too numerous to name or even remember, may I express my thanks for permitting me to obtain my intended goal as researcher and teacher of Veterinary Physiology.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>11</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>ILLUSTRATIONS</td>
<td>viii</td>
</tr>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. REVIEW OF LITERATURE AND THEORY</td>
<td>6</td>
</tr>
<tr>
<td>General Hemodynamics of Mitral Insufficiency</td>
<td>6</td>
</tr>
<tr>
<td>Theory of Possible Methods for Detecting and Quantitating Mitral Regurgitation</td>
<td>9</td>
</tr>
<tr>
<td>&quot;Strains&quot; for &quot;Stresses&quot; Generated by Mitral Insufficiency</td>
<td>11</td>
</tr>
<tr>
<td>Methods for Detecting Left Ventricular Hypertrophy</td>
<td>12</td>
</tr>
<tr>
<td>Methods for Detecting Vibration of Mitral Leaflets</td>
<td>15</td>
</tr>
<tr>
<td>Methods of Detecting Alterations in Pressure-Volume Relations</td>
<td>17</td>
</tr>
<tr>
<td>Pressure Fluctuations within the Left Atrium--Normally and with Mitral Insufficiency</td>
<td>21</td>
</tr>
<tr>
<td>Methods of Detecting the Shuffling of Blood across the Mitral Orifice</td>
<td>23</td>
</tr>
<tr>
<td>Normal Indicator-Dilution Curve</td>
<td>25</td>
</tr>
<tr>
<td>Cardiac Output Calculations from Indicator-Dilution Curves</td>
<td>41</td>
</tr>
<tr>
<td>Calculation of Central Venous Volume from Indicator-Dilution Curves</td>
<td>43</td>
</tr>
<tr>
<td>Indicator-Dilution Methods for Quantitating Mitral Regurgitation</td>
<td>45</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>III. PLAN OF RESEARCH</td>
<td>60</td>
</tr>
<tr>
<td>IV. MATERIALS AND METHODS</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>62</td>
</tr>
<tr>
<td>Animals</td>
<td>62</td>
</tr>
<tr>
<td>Preparation</td>
<td>63</td>
</tr>
<tr>
<td>Simultaneous Indicator-Dilution Curves</td>
<td>76</td>
</tr>
<tr>
<td>Experimental Groups</td>
<td>79</td>
</tr>
<tr>
<td>Analysis of Data</td>
<td>80</td>
</tr>
<tr>
<td>V. RESULTS</td>
<td>83</td>
</tr>
<tr>
<td>Simultaneous Indicator-Dilution Curves from Normal Dogs</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>85</td>
</tr>
<tr>
<td>Indicator-Dilution Curves from Dogs with Mitral Regurgitation</td>
<td>85</td>
</tr>
<tr>
<td>Simultaneous Indicator-Dilution Curves before and after Angiotensin II</td>
<td>91</td>
</tr>
<tr>
<td>Simultaneous Indicator-Dilution Curves from the Same Site in the Vascular System Sampled through Different Catheters</td>
<td>103</td>
</tr>
<tr>
<td>VI. DISCUSSION</td>
<td>107</td>
</tr>
<tr>
<td>VII. CONCLUSIONS</td>
<td>115</td>
</tr>
<tr>
<td>VIII. SUMMARY</td>
<td>117</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>120</td>
</tr>
<tr>
<td>AUTOBIOGRAPHY</td>
<td>125</td>
</tr>
</tbody>
</table>
TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Slope Ratios for Normal Dogs ..........</td>
<td>90</td>
</tr>
<tr>
<td>2. Slope Ratios for Dogs with Mitral Regurgitation ..................</td>
<td>90</td>
</tr>
<tr>
<td>3. Slope Ratios for Normal Dogs Before and After Angiotensin-II ................</td>
<td>96</td>
</tr>
<tr>
<td>4. Effects of Angiotensin-II on Various Parameters of the Cardio-vascular System ........</td>
<td>98</td>
</tr>
<tr>
<td>5. Slope Ratios of Curves Inscribed with Sampling from Same Site in the Aorta, but Sampling done through Cardiac Catheter and Courand Needle ........................................</td>
<td>104</td>
</tr>
</tbody>
</table>
# ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Illustration Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Indicator-dilution curve from a normal dog</td>
<td>27</td>
</tr>
<tr>
<td>2.</td>
<td>Schematic drawing of vessel showing profile of indicator particles</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>Schematic drawing of vessel showing profile of indicator particles</td>
<td>34</td>
</tr>
<tr>
<td>4.</td>
<td>Schematic drawing of vessel showing profile of indicator particles</td>
<td>37</td>
</tr>
<tr>
<td>5.</td>
<td>Effect of mitral regurgitation on dispersion of indicator particles</td>
<td>40</td>
</tr>
<tr>
<td>6.</td>
<td>Schematic representation of cardiovascular system</td>
<td>47</td>
</tr>
<tr>
<td>7.</td>
<td>Phonocardiogram from dog with mitral regurgitation</td>
<td>65</td>
</tr>
<tr>
<td>8.</td>
<td>Dorso-ventral radiograph of thorax from dog with mitral regurgitation</td>
<td>67</td>
</tr>
<tr>
<td>9.</td>
<td>Angiocardiogram from dog with mitral regurgitation</td>
<td>69</td>
</tr>
<tr>
<td>10.</td>
<td>Heart from dog with mitral regurgitation</td>
<td>71</td>
</tr>
<tr>
<td>11.</td>
<td>Color photograph of same heart viewed in preceding figure</td>
<td>73</td>
</tr>
<tr>
<td>12.</td>
<td>Enlargement of mitral valve area from same heart as in figures 10 and 11</td>
<td>75</td>
</tr>
<tr>
<td>13.</td>
<td>Indicator-dilution curves from the main pulmonary and femoral arteries of ten normal dogs</td>
<td>87</td>
</tr>
<tr>
<td>14.</td>
<td>Indicator-dilution curves from the main pulmonary and femoral arteries of five dogs with clinical evidence of mitral regurgitation</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 15. Slope ratios plotted against severity of mitral valve damage as estimated at post mortem .... 93

16. Indicator-dilution curves from the main pulmonary and femoral arteries taken before and after the administration of angiotensin-II. Arterial blood pressure recording appears at the top ...................... 95

17. Plot of per cent change in central venous volume against per cent change in slope ratio and per cent change in cardiac output ............................................. 100

18. Plot of per cent change in slope ratio against per cent change in cardiac output ............... 102

19. Simultaneous indicator-dilution curves from the same site in the aorta in normal dogs .... 106
CHAPTER I

INTRODUCTION

Constancy of the *milieu interne* results from a
dynamic equilibrium between the cells comprising the
organism and the external environment. In the mammalian
body this equilibrium depends upon the circulation of blood
within the cardiovascular system. In the blood are dissolved
solute transported either to or away from the body cells.
Transfer of these solutes may be attributed to: (1) motion
of blood through the vessels from points in proximity with
the body cells, and (2) either active or passive transfer
between compartments comprising the organism.

The major part of the energy for transfer of blood
and its contained solutes is derived from contraction of the
heart. Contraction of the heart results in the ejection of
a portion of its contents into the arterial system and the
reception of a portion of the blood from within the venous
system—the two proportions comprising equal volumes.

Effective conversion of energy by the heart into
kinetic energy of blood flow and into potential energy
stored in the elastic arteries is dependent upon uni-
directional flow of blood through the heart and vessels.
The valves within the heart prohibit bidirectional flow to
any significant degree. In particular, the mitral valve
regulates the direction of blood flow between the left atrium and ventricle.

In the dog (51), approximately 0.01 seconds after the onset of ventricular contraction, hydrostatic pressure within the left ventricle exceeds hydrostatic pressure within the left atrium. At this instant (slightly before in cases with long periods of diastole and slightly after in cases with short periods of diastole), the leaflets of the mitral valve are "washed" into apposition. This constitutes closure of the mitral valve and terminates patency of the mitral orifice. Pressure within the left ventricle rises during the subsequent 0.05 seconds as tension increases in the fibers comprising the myocardium. During this period, pressure within the left ventricle is inadequate to open the aortic valve against pressure within the aorta, but is adequate to maintain mitral valve closure against pressure within the left atrium. The left ventricle remains a closed cavity. Immediately following this period, as pressure within the left ventricle continues to rise, the aortic valve is opened by the dominant left ventricular pressure, and blood flows "down" the pressure gradient from left ventricle into aorta. The period during which blood is ejected from the left ventricle into the aorta lasts approximately 0.20 seconds; and, at this time, the walls of the arterial system are distended due to the influx of blood into the lumina they form. This distention constitutes the store of
potential energy which is destined to maintain the flow of blood through the vessels and to contribute to the net pressure (a resultant of hydrostatic and osmotic pressure) gradient which is responsible for the transfer of solutes from blood into and through cell membranes.

During ventricular contraction (systole), the atrio-ventricular annulus is displaced toward the cardiac apex, thus expanding the atrial lumen, decreasing the pressure within, and providing a portion of the \textit{vis a fronte} which facilitates filling of the heart for the subsequent contraction.

If for any reason the mitral valve fails to terminate patency between left atrium and left ventricle during the periods when left ventricular pressure exceeds left atrial pressure, a portion of the left ventricular contents will flow retrograde (regurgitate) through the mitral orifice into the left atrium. As will be discussed subsequently, many interesting phenomena determine the precise volume of blood which will regurgitate; but, under conditions of insufficient closure of the mitral valve, a portion of the blood which would have contributed to a greater increment in potential energy (mediated through mechanical deformation of the elastic structures in the arterial system) now travels retrograde and decreases the \textit{vis a fronte} (raises left atrial pressure).
Thus normal physiological activity which depends upon adequate conversion of energy of cardiac contraction to energy of blood flow and arterial distention may fail. Such failure may be termed "heart failure"; thus, inadequate closure of the mitral valve may cause the entity called "congestive heart failure" observed so frequently in aged dogs with clinical evidence of mitral regurgitation.

Before one may say, assuredly, that mitral insufficiency, and the associated regurgitation of blood, is in any way related to the clinical entity of "heart failure," one must first demonstrate positive correlation between the presence of congestive heart failure and not only the presence of mitral insufficiency, but the degree of mitral regurgitation. It is possible that dogs with mitral regurgitation diagnosed by routine clinical procedures may not have mitral regurgitation; or, if mitral regurgitation exists, this may be either of insufficient magnitude to affect the animal clinically, or of mere coincidence with the clinical syndrome observed.

Thus one might justifiably seek a method applicable to the clinical situation for detecting and for quantitating the amount of blood regurgitating through a mitral orifice.

Numerous methods to quantitate and to semi-quantitate the degree of regurgitation have been proposed. These, along with a summary of the classical observations related to hemodynamic principles associated with mitral regurgitation
observed clinically in man and in dog, and induced in dog, will be reviewed in this paper.

The salient purpose of this paper, however, is to describe a method for semi-quantitating the degree of mitral regurgitation. Also, the role that certain effectors of the cardio-vascular system play in altering the efficacy of this method for detecting mitral regurgitation and for discriminating between mitral regurgitation and physiological states which might simulate the disease entity as detected by the method proposed, will be described.
CHAPTER II

REVIEW OF LITERATURE AND THEORY

General Hemodynamics of Mitral Insufficiency

One of the earliest studies of cardiodynamics in mitral insufficiency came, appropriately so, from the laboratory of Carl J. Wiggers, who first described precisely the mechanical events of the cardiac cycle in the normal dog. In 1922, he and Feil (52) described the pressure events of the cardiac cycle in dogs with varying degrees of mitral regurgitation induced by separating the leaflets of the mitral valve with a hollow sound. The degree of patency could be regulated by altering the depth that a plunger was inserted into the sound. Observations were limited to fluctuations of pressure within the left atrium, left ventricle, and aorta, and to fluctuations of volume (plethysmography) of the left atrium.

Contrary to both reason and current thinking, they observed little, if any, regurgitation during the period of isometric contraction, even though the intraventricular pressure exceeds the intra-atrial pressure. Most of the regurgitation occurred during the period of ejection and during the first 0.08 seconds of diastole. Why little or
no blood regurgitated during isometric contraction may be explained by the following: (1) The duration is so short (0.05 seconds); (2) The total duration is required to overcome the inertial forces of blood within the left ventricle; and (3) The mean difference of pressure between left ventricle and left atrium during the period is only approximately 30 millimeters of mercury. During the relatively lengthy interval (0.35 seconds) comprising the period of ejection and the first 0.08 seconds of diastole, intraventricular pressure dominates intra-atrial pressure by approximately 100 millimeters of mercury, the inertial forces of blood are overcome, and blood is ejected from the left ventricular lumen into both aorta and left atrium.

Since, in the case of mitral insufficiency, a portion of the left ventricular contents is ejected regrograde through the mitral orifice, proportionately less energy is expended toward exceeding the end-diastolic pressure within the aorta and permitting departition of the aortic valve; thus, one would anticipate prolongation of isometric contraction. However, the duration of isometric contraction is little (0.001 seconds), if any, prolonged. Although less energy is expended toward exceeding the end-diastolic aortic pressure (the pressure which, if exceeded, will result in opening of the aortic valve), this pressure is lower due to the reduced quantity of blood injected into the aorta during the previous
contraction. Reduction in end-diastolic pressure within the aorta parallels reduction in the ability of the left ventricle to expend energy to overcome the pressure, with the result that no increase in duration of work (isometric contraction) is necessary.

Wiggers and Peil observed, further, that durations of ejection and net forward flow of blood remain normal even in the presence of moderately severe degrees of regurgitation. This, they postulated, was mediated through Starling's law of the heart. The volume of blood which regurgitates during each contraction contributes to the *vis a tergo* and to the end-diastolic volume within the left ventricle. This volume, which is increased immediately preceding the subsequent contraction, generates a more vigorous contraction, a larger stroke volume, and the observed normalcy of ejection time and net forward flow of blood.

It must be mentioned, however, that one may not exclude an increase in the *vis a fronte* from mediating the increased tidal volume; for, by more active relaxation of the ventricle, one might postulate greater diastolic "suction." This may be corroborated by the characteristic rapid "y" descent in the atrial pulse curve from humans and from animals with mitral insufficiency (to be discussed).

Wiggers and Peil concluded that, so long as the myocardium was capable of ejecting the increased tidal volumes, and, so long as the portion of that increased tidal
volume that traveled antegrade was adequate to maintain the animal, pressures within the pulmonary circuit remained normal.

Theory of Possible Methods for Detecting and Quantitating Mitral Regurgitation

Methods used to detect and to quantitate the degree of regurgitation through an incompetent mitral valve are based upon the detection of altered morphology and/or physiology (anatomical and/or physiological "strains") resulting from, and hopefully being proportional to, the degree of mitral regurgitation (physiological "stress"). In the case of an incompetent mitral valve, when the left ventricular pressure rises and exceeds both atrial and aortic pressures, proportions of the total left ventricular stroke volume will travel antegrade into the aorta and retrograde into the left atrium, the proportion in each direction being determined by the resistance to flow. Obviously, the facility with which the left ventricle may transfer contents of the pulmonary circuit into the arterial system will be impaired. Further, since the amount of work the ventricle performs equals, approximately, the volume it pumps times the average resistance against which it pumps, the work of the left ventricle would be increased. In the case of regurgitation and if the arterial system must be perfused with an amount of blood minimal for existence, the left ventricle must eject a greater than normal volume.
While it is true that the proportion of blood ejected retrograde is against the relatively low resistance in the left atrium, the work performed to eject this volume does contribute appreciatively to the work of the left ventricle.

Another sequel in regurgitation results from the incomplete closure of the mitral orifice by the valve leaflets. Blood is forced through this incomplete closure at a high velocity—velocity being proportional to volume flow per unit time and inversely proportional to cross sectional area. When the blood reaches the high velocities during traversal of the narrowed mitral orifice, the flow changes from laminar to turbulent.

Thus, the single "stress," mitral regurgitation, may be redefined in terms of three "types of stress"; (1) increased workload on the left ventricle, (2) increased velocity of blood flow through the narrowed mitral orifice, and (3) ineffective transfer of blood from the pulmonary circuit to the arterial circuit characterized by the shuffling, antegrade and retrograde, of blood across the incompetent valve.

If these "stresses" generate "strains" (reactions of the body) which, under certain conditions, may be detected by the observer they might become signs of their stress; and, further, their magnitude might parallel the magnitude of their stress (degree of regurgitation). The qualifying term "might" has been inserted purposely since, at least for
some of the theoretical strains attributed to mitral regurgitation, we know they do not parallel the degree of the stress and in fact may be induced by stresses completely unrelated to mitral regurgitation. For example, the observer may detect the result of increased work load by the left ventricle; but he may never deduce the particular stress (aortic stenosis, patent ductus arteriosus, mitral insufficiency) for which the strain was the reaction.

"Strains" for the "Stresses"
Generated by Mitral Insufficiency

Ineffective transfer of blood from the pulmonary circuit to the arterial tree and the shuffling back and forth of the blood between the two circuits, would result, theoretically, in an increased volume of blood and pressure within the pulmonary circuit, and decreased volume and pressure in the arterial circuit. The shuffling antegrade and retrograde of blood between the left atrium and ventricle would result in a further mixing of any foreign particles which may have been injected into the blood stream proximal to the "shuffling chamber" (mitral orifice) and left atrium, if the dispersion of the particles is analyzed "downstream" from the shuffling chamber.

Increased work load results in myocardial hypertrophy. This is consonant with the observation that a worker's biceps are larger (more hypertrophied) than those of the sedentary office worker.
Increased velocity of blood flow and turbulence of blood flow through the mitral orifice results in vibration of the mitral valve leaflets and possibly of the walls comprising the cardiac chamber.

The problem of detecting the degree of regurgitation is relegated, theoretically, to methods for detecting the degree of strain; i.e., (1) the amount of cardiac hypertrophy, (2) the degree of vibration of the mitral leaflets and/or walls of the heart, (3) the increase in volumes and pressures within the pulmonary circuit and decrease in volumes and pressures within the systemic circuits, and (4) the magnitude of the shuffling back and forth of blood in the mitral region.

As shall be pointed out, the degree of strain as measured by alteration in any given physiologic or anatomic parameter need not be always proportional to the magnitude of the stress.

Methods of Detecting Left Ventricular Hypertrophy

Aside from direct visualization and measurement of the thickness of the walls with calipers, radiography—in particular, contrast radiography—is the most valuable method for determining the presence or absence of ventricular hypertrophy (43, 44). Routine radiography of the thorax will permit assessment of total heart size and possibly preponderance of the left ventricular contribution.
to the cardiac silhouette; however, differentiation between left ventricular hypertrophy and left ventricular dilatation is impossible. The same is true with respect to the left atrium. Further, gross displacement of the heart or enlargement of the contralateral chambers may simulate ventricular hypertrophy.

Abelmann et al. (1) conclude that, in their experience, definite left ventricular enlargement or hypertrophy diagnosed with radiographs from patients with mitral valve disease without aortic disease, hypertension or mild cardiac failure (other stresses which may result in left ventricular hypertrophy) strongly suggests the presence of mitral regurgitation. They further suggest that inability to make a diagnosis of left ventricular enlargement or hypertrophy by the radiograph makes significant mitral regurgitation unlikely without ruling it out. Brigden and Leatham (3) conclude, similarly, that left ventricular enlargement is frequently present in cases of mitral insufficiency. Both groups of workers conclude that radiography is poor as a sole technique for detecting mitral regurgitation and is impossible as a method for quantitating the degree of mitral regurgitation.

No controversy exists over the efficacy of detecting left ventricular hypertrophy by selective chamber contrast radiography. If a radiographic exposure of the thorax is
made during the time when the left ventricular lumen contains radiopaque medium, the resulting radiograph will show, well opacified, the left ventricular lumen; and, less well opacified (but still more opaque than the lungs), the entire heart. One may "subtract" the opaque left ventricle from the entire silhouette and determine the thickness of the myocardium comprising the left ventricle. Such techniques, used for many years in human cardiology and recently in dogs, are effective for their purpose, i.e., detecting thickness of the left ventricle; but, to reiterate, they do not detect the cause of the left ventricular hypertrophy.

The electrocardiograph is a tool of extreme value in human cardiology for detecting and for semi-quantitating left ventricular hypertrophy (4). As the volume of muscle comprising the left ventricle enlarges during ventricular excitation, larger boundaries between resting and active portions of the left ventricle are formed which: (1) increase the magnitude of body surface potentials, (2) prolong their duration, and (3) reorient their "electrical axes" to be more parallel to the perpendicular constructed through the volume of muscle from endocardium to epicardium--the direction that the boundary of excitation moves.

In the dog, great variation in body configuration and location of heart within the thorax precludes use of
the electrocardiograph as an instrument for detecting left ventricular hypertrophy, at this time (21).

Methods of Detecting the Vibration of the Mitral Leaflets

The vibration of the mitral leaflets will occur when the orifice they form is inadequate in area to permit the blood flowing through it to retain laminar characteristics. When the flow converts from laminar to turbulent (according to the concept of Osborn Reynolds), the structures conveying the liquid begin to vibrate. If the vibration is of audio-frequency, it may be detected with a stethoscope as a "murmur"; if the vibrations are of a frequency to which the pressor receptors of the fingers are sensitive, it may be detected by palpation as a "thrill."

Such conditions of turbulent flow exist during ventricular contraction when the left ventricular pressure exceeds the left atrial pressure and when the leaflet of the mitral valve close imperfectly, providing a narrowed orifice through which blood regurgitates. Temporally this duration includes the periods of isometric and isotonic contraction and relaxation, although, as was discussed previously, little regurgitation occurs during isometric contraction.

The intensity of the murmur is proportional to the amplitude with which the valve leaflets vibrate, and to some extent their frequency (the human ear hearing certain frequency vibrations with more efficiency than those of
other frequencies). Factors determining the amplitude of vibrations are: (1) physical characteristics of the vibrating structure (the valves, chordae tendineae, and possibly the chambers), (2) the volume of blood traversing the narrowed orifice per unit of time, and (3) the cross sectional area of the orifice.

The intensity of the murmur is not necessarily proportional to the degree of stenosis. Moedjono et al. (37) who induced pulmonic stenosis of varying degrees in dogs showed that both intensity and duration of the murmur increased in proportion to the degree of stenosis, up to the time where the vessel lumen was approximately 35 per cent of normal. At this point the intensity of the murmur decreased. The same authors demonstrated further that, during certain phases of constriction, flow per unit time decreased, velocity of blood flow increased and the intensity of the murmur decreased.

Thus the value of the intensity of the murmur for estimating the degree of the regurgitation would be open to serious criticism. Leatham (31), in an excellent summary on systolic murmurs, described the pansystolic murmur of mitral insufficiency. It is accepted that, with few exceptions, mitral insufficiency may be detected—but not quantitated—by auscultation of the typical murmur.

In dogs, the murmur characteristic of mitral insufficiency is harsh, blowing, holosystolic, and
auscultated with maximal intensity at the left sixth intercostal space, one-third of the ventro-dorsal distance from the sternal (17). It is transmitted dorsally and caudally. The intensity may vary from grade one to grade five. In time, it extends from the first heart sound to the second heart sound, often occluding both. The murmurs of tricuspid insufficiency and ventricular septal defect are easily confused with that of mitral insufficiency. As in human cardiology, some animals with a grade four to five systolic murmur characteristic of mitral insufficiency may have, according to post mortem findings, only minimal mitral valve disease, whereas other animals having a grade one to three systolic murmur may have a mitral valve damage to an extreme degree.

Methods of Detecting Alterations in Pressure-Volume Relations

Alterations in the pressure-volume relations within compartments of the cardio-vascular system may result from mitral regurgitation. These may be more difficult to record, and their significance more difficult to assess. Difficulty in interpreting pressure-volume relationships results from two unknown factors: (1) modulus of elasticity of the left atrium and pulmonary veins, and (2) end-diastolic volume within the left atrium and pulmonary veins.

On a priori bases it appears that elevation of left atrial and/or volume during ventricular systole should
parallel the degree of regurgitation through the mitral orifice. On closer examination, however, the increase in volume is a function of not only the amount of blood regurgitating (milliliters per stroke), but also the per cent increase in left atrial end-diastolic volume and the compliance of the left atrium. Thus, if ten milliliters per stroke regurgitate into a left atrium comprised of relatively rigid walls and containing one hundred milliliters of blood, the atrium will undergo only a small per cent increase in volume. If, on the other hand, ten milliliters per stroke regurgitate into a volume of twenty milliliters contained in a chamber with relatively elastic walls, a large percentage increase in volume would occur. Thus, in both instances, the amount of blood regurgitating may be the same, but the changes in volume of the atrium into which the regurgitation occurs may vary in terms of per cent of initial volume.

With respect to increase in pressure paralleling the degree of regurgitation, although it is true that the change in pressure is proportional to the degree of regurgitation, the elasticity of the vessel containing the blood influences this parameter greatly. Obviously, if the walls of the left atrium are inelastic, even a small amount of regurgitation would increase the pressure; whereas, if the walls were elastic (the chamber has great "capacitance"), a large volume of regurgitation would increase the pressure only slightly.
Alterations in pressure and volume may indicate the degree of regurgitation, but they need not necessarily do so. Only when one knows, rather precisely, not only the pressure within the chamber, but also the end-diastolic volume and capacitance, may regurgitation be correlated with pressure.

Changes in volume within the left atrium which may be correlated with the degree of regurgitation may be studied by radiographic means. Fluoroscopically, systolic expansion of the left atrium has been observed in many human patients with marked mitral regurgitation. Elkin et al. (11) have shown that systolic expansion of the left atrium in patients with mitral regurgitation was falsely positive in 40 per cent of the cases, and falsely negative in 20 per cent.

In approximately 80 per cent of patients with mitral insufficiency, the atrial border electrokymogram showed a positive systolic plateau. This same plateau, however, occurred frequently in cases with pure mitral stenosis and in patients with no known heart disease.

Abelman et al. (1) describe certain factors which may conceivably contribute to the formation of the so-called "false" plateau tracings. They point out the importance of the elastic properties of the chamber, and the fact that inflammatory changes in the atrial wall, resulting from the mitral regurgitation, may alter the distensibility of the
atrium. They point out, further, that the atrium cannot be considered as an isolated chamber, since it is continuous with the pulmonary venous system. Wiggers (51) has shown that in the normal dog, the smaller left atrial and pulmonary venous system has a greater volume-elasticity coefficient (less distensible) than the left atrial-pulmonary venous system of larger dogs. He discussed the fact that, as a result of mitral insufficiency, the volume-elasticity coefficient of the atrium may be altered greatly as it enlarges and the walls become thinner and more distensible. Thus, it may be possible that a small amount of regurgitation into a small atrium may produce no greater systolic expansion than one might observe with the same amount of blood entering a large atrium, if the latter chamber is more elastic and one may assume continuity between left atrium and pulmonary veins.

In addition to fluoroscopy and electrokymography the volume of the left atrium may be determined by angiocardiography. Serial radiographs of the thorax may be taken as radiopaque material travels through the heart and its great vessels, outlining the respective chambers. A marked systolic enlargement may be observed in the left atrium of the animal with mitral insufficiency; however, in this instance also, the degree of expansion is influenced by factors other than the absolute volume of blood regurgitating into the left atrium.
Pressure Fluctuations Within the Left Atrium—Normally and with Mitral Insufficiency

Normally, three distinct peaks occur on the pressure curve recorded from within the left atrium during the cardiac cycle (51). The "a" wave is caused by contraction of the atrium. The "c" wave, occurring during isometric contraction, results from the closure of the atrio-ventricular valves and their evulsion into the atrial cavity. The "v" wave results from the *via a tergo*. The "x valley" occurs between the "a" and "c" waves and is caused by relaxation of the atrium. The "x valley" between the "c" and "v" waves is the sharpest descent in the pressure curve and is caused by the apical movement of the atrio-ventricular annulus. The "y valley" occurs between the "v" and "a" waves and is caused by the influx of blood into the ventricle from the atrium. The descent of the first portion of the pressure curve from the nadir of the "v" wave results from the rapid filling of the left ventricle. Normally, the "a," "c," and "v" waves do not exceed 5 millimeters of mercury, and "x" and "y valleys" may approximate -3 mm of mercury.

In mitral insufficiency, characteristically but not necessarily, the amplitude of the "c" wave is increased only slightly, the "v" wave becomes extremely great in magnitude and duration, and the "y" descent becomes extremely rapid (16, 34, 38). The increased pressure during the "v" wave represents the regurgitant blood filling the left
atrium during left ventricular ejection and the isometric relaxation. The rapid "y" descent reflects, possibly, an increased vis a fronte. The left ventricle must "over-fill" at the expense of the left atrium in order to eject a near constant forward output, and this is accomplished by greater diastolic suction (a larger left atrial-left ventricular pressure gradient) during the rapid filling phase.

Neustadt et al. (38) summarized the results of left atrial pressure curve from 43 patients—some with "pure" mitral stenosis, some "pure" mitral insufficiency, and some with combined lesions. They concluded that "the most useful feature of the left atrial pulse was the rate of the "y" descent in its initial 0.1 second, related to pressure at the v point (0.1 Ry/v) or to mean left atrial pressure." They also pointed out the "catheter washout" observed during left heart catheterization is of value. This refers to the fact that when a flexible catheter is inserted through the left atrium into the left ventricle, during systole it is often "washed back" into the left atrium with the regurgitant stream of blood.

The increased amplitude and duration of the "v" wave and the rapid "y" descent are characteristics of left atrial pressure curves in both man and dog with mitral insufficiency. Only in instances of a failing heart will the pulmonary arterial and right ventricular pressures be raised as a result of "v" wave transmission through the lungs. Similarly, only in the failing heart will an aortic
pressure be decreased, for, as was pointed out by Wiggers and Feil (52), net forward flow of blood is kept relatively normal due to compensatory mechanisms of the left ventricle.

It must be remembered as in the case of fluctuations in volume that the pressure fluctuations must be correlated with the elasticity of the chambers as well as the volume of blood within.

Gorlin et al. (16), Ross et al. (45), and Marshall et al. (34), in very excellent and comprehensive reviews of hemodynamic changes in mitral insufficiency, all agree that fluctuations in pressure within the left atrium may not be correlated in many cases with the degree of regurgitation through an incompetent mitral valve. All investigators agree that the rate of the "y" descent during the first 1/10 of a second from the nadir of the "v" wave is a valuable—if not the most valuable—indication of mitral insufficiency.

Methods of Detecting the Shuffling of Blood Across the Mitral Orifice

The antegrade and retrograde shuffling of blood across an incompetent mitral valve may be detected and possibly quantitated by injecting particles, foreign to blood but able to mix homogeneously with it, into a site "upstream" (intravenously) from the valve and determining the dispersion "downstream" (intra-arterially). A plot of the changes in concentration of foreign particles in the arterial blood following intravenous injection is termed an indicator-
dilution curve, and is one convenient method for expressing the dispersion of particles.

The dispersion is dependent upon three factors:
(1) the amount of blood flowing past the site of injection and all sites in the venous circulation temporally equidistant from the heart, (2) the volume of blood contained between the site of sampling and injection, and all sites temporally equidistant from the heart, and (3) certain peculiar "randomizing properties" of the system through which the blood and indicator particles flow. Sheppard (48) has summarized the degree of dispersion as being a function of "fraction of bed displaced per unit time" and "randomizing power of the bed." The "bed" refers to the volume of blood between site of sampling and injection, and all temporally equidistant points; "the fraction of the bed displaced per unit time" refers to the ratio of cardiac output to volume of the bed; and "randomizing power" results from factors within the volume that tend to further mix the indicator and blood. Examples of factors which tend to further disperse (randomize) the particles are: turbulence of flow, arterial-venous communications, and mitral insufficiency, in which case the particles and blood shuffle to and fro across the mitral valve permitting further mixing and retardation of a portion of the mixture. Thus mitral regurgitation may cause a dispersion of indicator particles
greater than that which would be anticipated under conditions of normal circulation.

**Normal Indicator-Dilution Curve**

The following paragraphs will be devoted to a discussion of the normal indicator-dilution curve: the configuration, the nomenclature expressing its parameters and, most importantly, the factors, normal and those present in mitral regurgitation, which determine its configuration. Immediately following will be a discussion of various modifications of the indicator-dilution method which have been used to estimate the degree of mitral regurgitation.

An indicator-dilution curve typical of that obtained from a normal dog appears in Figure 1. The concentration of the indicator within blood at the sampling site is plotted on the ordinate, and one-second time intervals are marked by the major vertical line and by time pips along the abscissa.

The indicator is injected intravenously at the instant i. At the instant A the first, most rapidly moving, particles of indicator which take the most direct path, reach the sampling site in the femoral artery. The duration between time of injection and time of arrival of the most rapidly moving particles is termed the arrival time (AT). The concentration of indicator in blood then undergoes a rapid increase between the time of arrival of the first particles and the time when the concentration reaches a peak.
An indicator-dilution curve recorded from a normal dog. The concentration of indicator in blood is plotted along the ordinate, calibrated to the right, and the time following injection is plotted along the abscissa, each major vertical line occurring every one second. \( A \) represents the point of injection of the indicator. \( AT \) represents the duration between injection of indicator and the time when the first particles arrive (A) at the point of sampling. \( BT \) represents the duration during which the concentration of indicator-in-blood decreases from the time of arrival (A) until the concentration reaches its peak (PC). \( DT \) represents the duration during which the concentration of indicator-in-blood increases from its peak concentration (PC) to the point where the most rapidly moving particles return—after traversing the circulation once—to the site of sampling, and will cause an increase in concentration. \( LC \) represents the point on the curve between the peak concentration of the first circulation of indicator particles and the peak concentration of the recirculation peak (RP). Below and paralleling the abscissa of the indicator-dilution curve is a section of arteries from which the blood samples may have been taken. Dots represent particles of indicator. The dispersion of the indicator particles from the "square wave" profile that is injected into the vein may be observed in the artery. Such a "distorted" profile results from two factors: (1) the particles tend to travel in the center of the stream, with the particles located centrally moving most rapidly and the velocity decreasing the more peripherally they are, and (2) some particles take longer pathways through the cardiac-vascular system than others.
This duration is called the build-up (BT) and the peak is called the peak concentration (PC). After the peak, the concentration falls gradually. The decay in concentration of particles traversing the arterial tree for the first circulation is interrupted by an increase in concentration caused by those more rapidly moving particles which have traversed the entire circulation and returned for recirculation at the sampling site. The duration between the time of peak concentration and the time when the first recirculation beings is termed the decay time (DT). Following the decay time, an increase in concentration develops a second peak (RP) on the curve which represents the first recirculation of indicator which has traversed the entire circulatory system. The lowest concentration represented on the dilution curve between the peak concentration and the recirculation peak is called the "least concentration." The peak of recirculation is of lower magnitude than the peak representing primary circulation of indicator, and is followed by a third peak, which represents the second recirculation of indicator. This is of still lower magnitude. Following the peak of the second recirculation the indicator is nearly homogeneously distributed within the blood, and little fluctuation in concentration of indicator in blood occurs. A slight but steady decrease in concentration results from the excretion of indicator from the body.
For nomenclature of parameters used in the interpretation of indicator dilution curves the reader is referred to the glossary of Wood and Swan (54).

Hamilton et al. (18) demonstrated in models without recirculation that the decay in concentration of indicator in blood is logarithmic. Taking advantage of this fact, they were able to define a curve representing only the first circulation of indicator uninterrupted by subsequent circulations. This they accomplished by plotting the first few points on the decay slope (before recirculation has occurred) on semi-logarithmic paper. These points establish a straight line (representing the logarithmic decay in concentration) which may be extrapolated to one-tenth per cent of peak concentration. The entire curve so plotted on semi-logarithmic paper represents an aliquot of nearly all of the indicator injected. The total duration under the curve represents the total time required for nearly all of the aliquot to traverse the sampling site for the first circulation, and also represents the total time required for nearly all of the indicator to traverse all possible arterial sampling sites equidistant from the heart with the femoral artery.

To aid in understanding the true genesis of the indicator-dilution curve, a hypothetical segment of artery from which the samples are taken appears below the abscissa of the graph in Figure 1. Within the artery shading appears
which represents the concentration of indicator particles at many time-segments during inscription of the indicator-dilution curve. The profile to which a "square wave bolus of indicator" is distorted by characteristics of the circulation is apparent. The particles congregate in an area of greatest concentration bounded at the head by a few particles which travel either in a faster moving portion of the bloodstream or through shorter pathways than the dominant portion of indicator. The greatest concentration of particles is bounded in the rear by a "tail" comprised of particles which travel either in a slower-moving portion of blood or via longer pathways.

Particles comprising the recirculation of the more rapidly moving indicator particles are excluded.

Factors which disperse particles to produce the profile observed in the schema below Figure 1 are further schematized in Figures 2, 3, and 4. As is shown in Figure 2, if the indicator is injected only slightly "upstream" from the site of sampling, little distortion in distribution profile exists over that generated by the method of injection; and the indicator dilution curve recorded from the point of sampling is similar to that which would have been recorded at the point of injection.

In Figure 3, which simulates more accurately the normal circulatory system, indicator particles may traverse any of three paths, but unite to form a final common path
Figure 2

Schematic drawing of vessel into which indicator particles are injected at point 1 and blood and indicator mixture is sampled at point s. Direction of flow, indicated by the arrow in the center of the vessel, is from left to right. The straightness of the arrow represents laminar blood flow. Configurations to which the bolus of particles are diluted appear at either extremity. Above each bolus profile appears schematic indicator-dilution curves (C = concentration, T = time) typical of what one might expect if sampling were at respective points in the circulation. Notice the similarity in indicator-dilution curves of bolus profile at the two sites. This suggests little added dispersion of indicator particles between the points of injection and sampling.
Same schema as in previous two figures with the exception of three possible routes for blood to travel. This schema approximates more closely the true vascular anatomy. Why the indicator particles would be more greatly dispersed than in the previous two schemas is obvious. Some particles will arrive at the sampling site via the most direct route, whereas other particles will arrive, more tardily, via less direct routes.
from which they are sampled. Although the same number of particles traverse points of injection and sampling, they are obviously more greatly dispersed at the point of sampling after having traversed the three possible routes. Particles comprising the head of the profile at the site of sampling were probably those particles which traveled the most direct route and in the center of the blood stream where the blood velocity is the greatest.

Particles in the most dense aggregate, immediately subjacent to the point of peak concentration in the indicator dilution curve, are probably those particles which moved more rapidly through the longer pathways and more slowly through the shorter pathways. The tail of the profile of indicator particles is comprised of those particles which moved most slowly through the longest pathways (the "stragglers"). As is shown in the next figure, 4, a similar dispersion of indicator particles may result from certain abnormal flow characteristics within a single tube even though the sites of injection and sampling are quite close. If certain obstructions occur within the system which tend to further agitate the mixture of dye and blood, the direction of flow of blood carrying certain of the particles will be altered, with the result that some of the particles carried will be retarded, the tail of the profile will be elongated and the peak concentration of the major aggregate of particles will be decreased.
Figure 4

Same schema as previous figure only with impediment placed in the vessel. The impediment changes laminar flow to turbulent flow (represented by spiraled arrow). Such turbulence agitates the indicator and blood mixture and results in further distortion of the profile. Notice the decreased peak concentration and prolonged decay slope in curve above right portion of the vessel as contrasted with that of left portion of vessel.
If one were to connect the system of tubes schematized in Figure 3 with that schematized in Figure 4 it would be quite obvious that if an indicator were injected into the inflow of the system and sampled at the outflow, the particles would be still more greatly dispersed, the peak concentration would be very low, and the tail of the curve extremely long. It is also true that the arrival time would be very little affected, since the more rapidly moving particles could still have taken a direct, unimpeded path through the center of the system of tubes.

Figure 5 is a reproduction of the very excellent schema proposed by Wood et al. (55) to represent the effect of mitral insufficiency on the dispersion of indicator particles injected proximal to the lesion and sampled distal to the lesion. If particles of the indicator were injected into the left atrium during ventricular diastole, they would be aspirated into the left ventricular lumen. During the subsequent ventricular systole, a portion of the indicator particles would be injected into the aorta antegrade, and into the left atrium retrograde. This would result in a decrease in concentration of indicator particles in the portion of the bolus with greatest concentration and a retardation of particles which had regurgitated into the left atrium. Thus, an indicator-dilution curve inscribed from sampling from the aorta would have a lower peak concentration and a longer tail than if the system had no
Figure 5

Classic illustration of the effect of mitral insufficiency on the dispersion of indicator particles within the heart. This illustration is copied from Wood (55) and is described thoroughly in the text. Indicator particles are injected in A. During ventricular systole, B, indicator particles are aspirated into the left atrium and diluted with a portion of the blood which has regurgitated into the left atrium. In ventricular diastole, C, indicator particles and regurgitated blood are aspirated into the left ventricular lumen. In the subsequent ventricular systole, D, a portion of the indicator and blood is ejected into the aorta and a portion is ejected regurgitantly into the left atrium. In the subsequent diastole, E, the ventricle aspirates the regurgitated indicator particles, from the left atrium. In the last ventricular systole, F, a fraction of the remaining portion of indicator particles within the left ventricle is ejected into the aorta and a fraction is ejected into the left atrium regurgitantly. An indicator-dilution curve inscribed from such a vascular system is shown as the solid line; the indicator-dilution curve from a normal system is shown with a dotted line. Why the peak concentration is lower and the decay slope of the curve is prolonged would be obvious from the illustration.
regurgitation. The amount of indicator which would have traveled retrograde through the mitral orifice, with respect to the amount which would have traveled antegrade through the aortic valve, would be proportional to the aliquot of the stroke volume which traveled in the respective directions. Thus, one would anticipate that the length of the tail (the slope of decay in concentration) and the degree to which the peak on concentration is reduced, would be proportional to the degree of regurgitation.

Cardiac Output Calculations from Indicator-Dilution Curves

Cardiac output may be estimated from indicator-dilution curves (9). The rectilinear presentation of the curve must first be transcribed to semi-logarithmic paper, and the decay slope extrapolated to one-tenth per cent of the peak concentration. The duration between arrival time and the time when the decay slope intercepts the line representing the one-tenth per cent of peak concentration includes the total time required for all of the indicator dispersed in blood to traverse the sampling site and all sites temporally equidistant from the heart. The average concentration of indicator in blood during inscription of the curve may be calculated by summing the concentration of dye in blood at each second during inscription of the curve and dividing the sum by the total number of seconds. The exact amount of indicator injected is known.
It now becomes possible to answer the question, How much blood, carrying the average concentration of dye determined from the curve, must have traversed the site of injection and all sites temporally equidistant from the heart in order to carry an aliquot of all of the indicator from the point of injection to the point of sampling in the time represented by the duration of the indicator-dilution curve? The area under the indicator-dilution curve represents only an aliquot of the total amount of the dye injected, for only the proportion of dye diluted in the fraction of the cardiac output that traversed the sampling artery could be detected. One may assume that the configuration and timing of an indicator-dilution curve inscribed from any other arterial sampling site would be identical with that inscribed from the site in question.

The situation is similar at the point of injection. Although all of the indicator is injected at one site in the venous system, the indicator-dilution curve inscribed at any arterial sampling site would be identical if the injection had been made into hundreds of venous sites, with the volume injected being proportional to the fraction of the cardiac output which returns to the heart through the given venous segment. As in the case with the many possible arterial sampling sites, the venous sites of injection must be all temporally equidistant from the heart.
Calculation of the cardiac output from an indicator-dilution curve becomes a modification of the direct Fick principle for estimating cardiac output, once one knows the average dye-carrying-capacity of the blood and the total duration required to carry all of the indicator particles by the sampling site. The cardiac output is equal to the total amount of dye injected divided by the average concentration times the total duration required for inscription of curve. If the amount of dye injected is in milligrams, the average concentration is in milligrams per liter and the time is in seconds, the cardiac output may be expressed in liters per minute by multiplying the quotient by 60.

\[ C.O. = \frac{I \times 60}{C \times t} \]

Calculation of Central Venous Volume from Indicator-Dilution Curves

The central blood volume (volume of blood between point of injection and sampling, and all points temporally equidistant from the heart) may be estimated by multiplying the cardiac output in milliliters per second by the time required for the average particle to traverse the volume between the injection site and sampling site. This duration is termed the mean circulation time or the mean transit time. It is estimated by multiplying the concentration of dye in blood at each second by the duration following the injection,
summing the products and dividing by the sum of the concentrations.

\[ CVV = \frac{C \cdot O}{60} \times MCT \]

Sheppard (48) described the postulates necessary for validating the theory of the indicator-dilution curve and the ability to estimate cardiac output from the curve, as follows:

1. All dye entering the circulating volume must be recovered.
2. The dye curve must provide a record equivalent to or proportional to the concentration of uniformly mixed samples at the outflow.
3. The indicator and substance being traced (blood) must behave identically.
4. The horizontal scale of the dye-dilution curve must be units of volume.
5. Concentration of label must be uniform over the entire cross section of fluid entering the system (minimal streamlining).

Since these postulates may be satisfied with little difficulty, cardiac output estimations from the indicator-dilution curve are accurate.

The central blood volume determination from the indicator-dilution curve are of questionable meaning; since,
as was pointed out by McIntosh et al. (35), the central blood volume is not an anatomical volume (i.e., the volume between points of injection and sampling and all geometrically equidistant points) but is a flow or dilution volume. That is, under conditions of constant cardiac output but altered segmental distribution of the cardiac output, grossly different central blood volumes may be calculated from the indicator-dilution curves, even though the anatomic central blood volume has remained constant. Some point in the arterial system temporally equidistant from the heart with respect to the sampling site may, in fact, be located in the venous system, because of the rapid flow of a large blood volume through a particular segment of artery (Figure 6). Nevertheless, Schlant et al. (46) compared a dye dilution method with a direct method for calculating central blood volume. The authors found that the dye dilution method overestimated the central blood volume by 12 per cent, systematically. One must always remember that the central venous volume is the volume available for dilution of the indicator.

Indicator-Dilution Methods for Quantitating Mitral Regurgitation

From the dispersion of indicator particles in given time-segments of an artery, one may estimate cardiac output and central blood volume. Conversely, under given conditions
Figure 6

A schematic representation of the cardiovascular system with veins (1, 2, and 3), pulmonary capillaries (5, 6, and 7), arteries (9, 10, and 11), the point of indicator injection (1), the point of sampling (s), and the right (4) and the left (8) hearts. Dotted lines (a, b, c, and d) are constructed at right angles to the mean axis of blood flow. Each line represents point on the vessels they intersect which are temporally equidistant from the heart. That is, all particles of blood in venous segments at which the lines intersect will arrive at the heart nearly simultaneously. Similarly, all particles of blood which leave the heart simultaneously will arrive in a segment of artery intersected by the dotted line d.

Although, in the illustration, the isochronic lines are constructed both straight and at right angles to the mean axis of blood flow, they need not have been. For it is feasible—and, in fact, correct—that the blood may flow more rapidly to certain segments. Thus, points located anatomically equidistant from the heart may not be so temporally.
of cardiac output and central blood volume, the dispersion of indicator particles in different time-segments of an artery may be predicted. The time of arrival of the most rapidly moving particles may be predicted, the height of the peak concentration may be predicted, and the slope of the decay in concentration may be predicted.

It is because of this predictability that indicator-dilution curves are valuable in detecting and possibly in quantitating mitral regurgitation. That mitral regurgitation affects the degree of particle dispersion has been established previously. Thus, if one observes, from an indicator-dilution curve, certain characteristics of particle dispersion that are incongruous with the expected normal conditions of flow and central blood volume, one may assume the action of a third factor. It must be granted that this third factor may be compounded of many; therefore, one must be cautious in asserting that the physical act of blood and dye particles shuffling back and forth across the mitral orifice is the only factor, in addition to flow and central blood volume, that affects the dispersion.

The following applications of the indicator-dilution method have been used to detect and to quantitate mitral regurgitation.

The first attempts at quantitating mitral regurgitation using the indicator-dilution method were by Korner and Schillingford (28), in 1955. They obtained
single indicator-dilution curves by injecting indicator into the right atrium and sampling from any peripheral artery. They observed that such indicator-dilution curves had "a lower peak concentration and a disproportionate prolongation of the disappearance slope" in patients with valvular incompetence. They observed, further, that either a low cardiac output or a large volume diluting the indicator produced similar alterations in the dilution curves. Their approach at quantitating the degree of regurgitation was a statistical one, based upon indication-dilution curves recorded from circulatory models in which varying degrees of regurgitation and alterations in flow and volume could be induced. Quoting from Carleton et al. (5), in their excellent treatise on the assessment of the Korner-Schillingford method,

They treated the dilution curve as a frequency distribution of dye particles, and regarded the effect of regurgitation as a diminished probability of forward movement of a given dye particle. They attempted to assess this probability as a function of the dispersion of the curve. Choosing either the variance or the reciprocal of the slope of the descending limb as the expression of dispersion, they demonstrated in their model that each of these consisted of a component largely determined by flow and another largely determined by the volume between the sites of injection and collection. With valvular incompetence, both the variance and the reciprocal of the slope increased and could be mathematically separated into components related to forward flow, volume, and regurgitant flow.

Since their studies with the model had shown that the conventional methods of calculation gave correct results for flow and volume whether or not regurgitation was
present, they could by means of a regression equation relating either the variance or the reciprocal of the slope to flow and volume, calculate the parameter expected in the absence of regurgitation. They then described the diminished probability of forward flow as the relation of the expected variance or reciprocal of the slope to the value observed from the dilution curve. Regression equations were calculated on normal individuals, i.e., those with no evidence of valvular incompetency. Slopes of the curves from the patients with mitral regurgitation are more gradual than those from normal patients. From the normal range of slopes and from the slope of the subject with mitral insufficiency, one may conclude that one standard deviation from the mean of curves from normal patients may include a slope as gradual as that from the abnormal patient in this instance.

Eich et al. (10) corroborated the observation that slopes from patients with mitral regurgitation fall within the limit of slopes recorded from normal patients. They studied mongrel dogs in which a left ventricular-left atrial shunt of varying degrees was inserted. Although, in each animal, the indicator-dilution curve during mitral regurgitation was more slurred than that before regurgitation, no ratios relating factors of time or concentration derived from the curves were of value in separating normal from abnormal curves. That is, the
indicator-dilution curve from the dog with mitral regurgitation in every case fell within limits considered normal for dogs.

Another very important objection to the detection and quantitation of mitral regurgitation by single indicator-dilution curves is that these curves detect only a shuffling back and forth of indicator and blood across a valve—not necessarily the mitral valve; therefore, tricuspid, pulmonic or aortic insufficiency may not be distinguished from mitral insufficiency.

Hoffman and Rowe (23), using circuit models of the circulation, have shown that varying the size, shape or elasticity of the chamber into which the blood regurgitates alters the parameters of the indicator-dilution curve such that the discrimination obtained by the Korner-Schillingford method is inadequate. Further, they pointed out that the "central blood volume" used by Korner and Schillingford in their calculation contained volumes of blood outside the limits described by the physical point of injection and sampling; therefore, even on theoretical grounds, regression equations calculated on this basis may be erratic. Carleton et al. (5) demonstrated in their review that the Korner-Schillingford method, using the variance, was sound if the calculated central venous volume more closely approximated the true mixing volume.
In 1957, Wood et al. (55) demonstrated that mitral regurgitation could be discriminated from mitral stenosis by a single indicator-dilution curve. They asserted that the ratio of the recirculation peak to the least concentration reflected the degree of regurgitation. The closer the ratio approached 1, the greater was the degree of regurgitation.

Schillingford (49), in 1958, and Resnekov (42), in 1961 attempted to quantitate the degree of mitral regurgitation by a "dispersion index." The index was calculated by dividing the spread of the indicator-dilution curve in time at the 10 per cent of peak concentration level by the arrival time. This was modified by Hannock (22) who expressed the degree of regurgitation by relating peak concentration to the time required for the dye to decrease to 10 per cent of the peak concentration.

It is apparent that these methods are modifications of the original Korner-Schillingford method. They all represent the decreased slope of the decay in indicator concentration in curves from subjects with mitral regurgitation as compared with slopes from curves in normal subjects.

It must be reiterated, however, that the wide variation in slopes from normal subjects includes, in many cases, slopes from patients with mitral regurgitation. Slopes from patients with mitral regurgitation of a severe
degree may not be confused with slopes from borderline normal subjects; however, in these cases indicator-dilution curves are superfluous. Furthermore, it has been demonstrated by many investigators that a marked increase in central venous volume or a marked reduction in cardiac output may mimic the slope from the patient with mitral regurgitation. Although the arrival time of indicator particles in the femoral artery is asserted to be normal or accelerated in patients with mitral insufficiency but prolonged in patients with mitral stenosis, it remains extremely difficult to assess the severity of either lesion if both are concomitant in a patient. In addition, even the slightest degree of congestive heart failure or stasis of circulation may produce an indicator-dilution curve with a slope identical to that observed in mitral insufficiency.

To circumvent objections to the Korner-Schillingford indicator-dilution method for detecting and for quantitating mitral regurgitation, two alternatives were described.

Keys, Swan and Wood (26), in 1956, proposed a method for quantitating mitral regurgitation by injecting an indicator into the left ventricle and sampling, simultaneously, from the root of the aorta and from the left atrium. In the individual without regurgitation, no indicator was detected in the left atrium during the
inscription of the primary curve recorded from the aorta. With mitral regurgitation, indicator was detected in the left atrium during this time. The proponent postulated that the ratio of the area under the curve inscribed from the left atrium to that inscribed from the aorta is as the fraction of the blood and dye mixture which regurgitated through the valve is to the net forward flow. Serious objections to this method have been suggested by the work of Irisawa (24) who pointed out that the left ventricle is not a "perfect" mixing chamber for the dye, and that it is possible that methods are not valid if based upon the assumption that uniform mixing of left atrial blood entering the left ventricle does occur.

Recently, however, Kuykendall et al. (29) have shown that, in dogs with varying degrees of mitral valve damage, the degree of regurgitation may be quantitated quite accurately. They showed further that the accuracy is not impaired by the site of injection within the left ventricle, by the time during the cardiac cycle when the injection is made, by the heart rate (within physiological limits), by the duration of injection, by the site of sampling within the left atrium or by left to right intracardiac shunts. They did show that a falsely low value for regurgitation may be obtained in the presence of premature ventricular beats. Furthermore, congestive heart failure, stasis of circulation, or reduced cardiac output should not, theoretically, affect
the efficacy of this technique. Objections to the use of this method for quantitating mitral regurgitation in dogs result from the necessity of inserting a needle into the left ventricle and catheterizing the left atrium—both rather formidable procedures in the dog.

In 1958, Lang and Hecht (30) published their method for detecting and for semi-quantitating the degree of mitral regurgitation by using indicator-dilution curves recorded simultaneously from the main pulmonary artery and from the femoral artery. Their site of injection was the right atrium. With this technique they hoped to obviate certain objections to the single indicator-dilution curve for quantitating mitral regurgitation. For example, when one records only a single indicator-dilution curve from an arterial site, congestive heart failure, stasis of blood flow, or left-to-right intra cardiac shunts may simulate mitral regurgitation as detected by the indicator-dilution curve. If, however, two indicator-dilution curves were inscribed, simultaneously, one between the site of indicator injection and the mitral valve and the other "downstream" from the mitral valve, many factors which may tend to mimic mitral regurgitation so far as indicator-dilution curves are concerned, would alter indicator-dilution curves from both sites in a similar fashion. Mitral regurgitation, on the other hand, would exert its effect on the dispersion of particles as detected only from the arterial sampling site,
but not from the main pulmonary artery sampling site. It must be conceded that by this technique aortic insufficiency could not be discriminated from mitral insufficiency. Thus, Lang and Hecht did not need to revert to regression equations to anticipate what the slope of the curve from the femoral artery from a particular patient under normal conditions might be, since the indicator-dilution inscribed with sampling from the main pulmonary artery served as this "control curve." Their method of estimating the degree of regurgitation with respect to net forward flow was to subtract the difference in arrival time between the two curves from the difference in mean circulation time between the two curves and divide this value by the difference in arrival time. This ratio represents the ratio of the regurgitant flow to the total forward flow. They suggest that the curves from the two sites should be nearly congruent, and that little—if any—further mixing of indicator with blood occurs between the sampling site in the main pulmonary artery and that in the femoral artery.

They demonstrate the value of this technique for discriminating mitral regurgitation from mitral stenosis in human subjects. Studies with physical models of the vascular system were conducted to show the theoretical basis for this discrimination.

Recently Wilson et al. (53) corroborated the value of this method for detecting and for quantitating mitral
regurgitation in human subjects with or without mitral stenosis. They considered it accurate in greater than 90 per cent of the patients studied.

Emanuel et al. (12) designed a series of experiments to elucidate the effectors of the rate of indicator decay in normal dogs and in dogs with mitral insufficiency, induced surgically. In the initial portion of the study, indicator was injected into the anterior vena cava and blood samples were taken, simultaneously, from the left atrium and from the aorta, and from the left atrium and from the femoral artery. Indicator-dilution curves recorded from the left atrium and from the aorta were sufficiently similar to suggest that the volume between the two sampling sites had little to do with the configuration of the indicator-dilution curve and that the volume between the site of injection in the vena cava and the site of sampling in the left atrium was paramount in determining the configuration of the indicator-dilution curve. This volume constitutes the volume of blood within the lungs. Indicator-dilution curves obtained from the femoral artery were deemed unreliable since the slope-volumes calculated from this site differed by an average of 21 per cent from that calculated from the left atrial sampling site.

When the authors induced mitral incompetency in five dogs, indicator-dilution inscribed from the left atrium and from the aorta remained nearly identical to each other, but
differed markedly from the control in that their slopes were more gradual and a peak concentration was lower. They attempted to explain the decrease in slope by a decrease in cardiac output following induction of mitral insufficiency; however, they assert that since the slope diminished out of proportion to the output, they must conclude that the volume of blood with which the indicator mixed (in the lungs) must have increased. They demonstrated that the decrease in slope from both sampling sites following injection into the anterior vena cava was in fact a result of the increased volume of blood in the lungs and was not a consequence of any added mixing of indicator with blood located in the heart. To do this, they injected indicator into the left atrium and sampled from the aorta. Their results showed that the indicator-dilution curves inscribed from the aorta following left atrial injection had, in every case, slopes steeper than those from curves obtained when injections were made into pulmonary arterial tree. Thus, since the same lesion (mitral insufficiency) was present irrespective of where the indicator was injected, and since the slope of the curve from the aorta was sharper when the indicator was injected in the left atrium than when it was injected into the anterior vena cava, they felt justified in concluding that it is the increased volume of blood within the pulmonary circuit that generated the decreased slope of the indicator-dilution curve in cases of mitral insufficiency.
Lukas, et al. (33), in a preparation identical to that used by Eich et al., recorded indicator-dilution curves from the aorta of dogs. The rate of regurgitation was altered from 0.9 to 3.3 liters per minute. The reciprocal of the down slope and the variance of the curves obtained following injection of indicator into the left atrium was a reliable indicator of the volume of blood regurgitating. When injections of indicator were made into the pulmonary artery, the amount of regurgitation was underestimated. In the hands of these investigators, this method was valuable for detecting varying degrees of mitral regurgitation; however, as was the case with Eich, the slope of curves from animals with all but the most severe regurgitation fell within limits of the slope of the normal dog.
CHAPTER III

PLAN OF RESEARCH

As has been reviewed in the previous section, many different methods of analyzing both single and double indicator-dilution curves have been evaluated as techniques for quantitating mitral regurgitation. This author believes that the methods of injecting into the left ventricle and sampling, simultaneously, from the left atrium and the aorta, and of injecting into the jugular vein and sampling, simultaneously, from main pulmonary and femoral arteries are the most valuable. The former method requires the rather difficult and potentially hazardous techniques of left atrial and aortic catheterization and of left ventricular puncture. The latter method requires only routine right heart catheterization and percutaneous femoral artery puncture.

This thesis contains results of attempts to quantitate the degree of mitral regurgitation by analysis of one simple relation between indicator-dilution curves inscribed with simultaneous sampling from main pulmonary and femoral arteries. Tangents to the decay in concentration will be drawn to the indicator-dilution curves inscribed
from both sites on rectilinear paper. The tangents will be
drawn either at the point one-fourth of the distance from
peak concentration to zero concentration or at the series of
points on the early portion of the decay slope where the
decay is apparently linear. A ratio of the slopes of the
tangents drawn to the main pulmonary artery curve and to the
femoral artery curve will be calculated. It is hoped that
this ratio will represent the added degree of indicator
particle dispersion generated by mitral regurgitation—that
is, the greater the degree of regurgitation, the greater the
ratio.

Research will be conducted in the following
categories:

1. obtaining facility with the apparatus and
methods for inscribing indicator-dilution curves
simultaneously,
2. determining the slope ratios for normal dogs,
3. determining the slope ratios for dogs with
clinical evidence of mitral regurgitation, and
4. evaluating the effects of altered central venous
volume and catheter sampling systems on the slope
ratios.
CHAPTER IV

MATERIALS AND METHODS

Animals

Thirty normal dogs used in this study were selected from the animal procurement facilities of The Ohio State University and the Martin Company, Denver Division. Normalcy was estimated from auscultation of the heart and lungs, by the presence of well-formed stools, by normal skin turgor, and by general clinical impression. Seven dogs with mitral insufficiency were donated by veterinarians or by the Veterinary Clinic of The Ohio State University.

The presence of mitral insufficiency was determined by:

1. A grade IV or louder holo-systolic murmur heard with maximal intensity at the left cardiac apex beat,
2. Radiographic evidence of left ventricular and left atrial enlargement,
3. Angiocardiographic evidence of opacification of the left atrium following injection of radiopaque material into the left ventricle,
4. Post mortem findings of:
   a. thickening and contraction or vegetation of leaflets comprising the mitral valve,
   b. left ventricular hypertrophy, and
   c. endocardial deposition of scar tissue in the left atrium adjacent to the deformed leaflet of the mitral valve.

Examples of the observations suggestive of mitral insufficiency are shown in Figures 7-12.

The normal dogs were mongrels between one and four years of age, between 10 and 15 kilograms in weight and were either mesomorphic or meso-ectomorphic. Both sexes were equally represented.

Dogs with mitral insufficiency were between 8 and 14 years of age, between 12 and 14 kilograms in weight, and were either mesomorphic or meso-endomorphic. Four were males and three were females. None demonstrated signs causally related to heart disease. Three had chronic coughs, five had polyuria, four had dermal tumors.

Preparation

Preparation of animals was similar in all instances. They were anesthetized intravenously with pentobarbital sodium, 25 milligrams per kilogram body weight. The right external jugular was exposed; and, under fluoroscopic guidance, a 50 centimeter 8F, NIH-tip cardiac catheter was introduced into either the left or the right pulmonary
Figure 7

Phonocardiogram of dog 5M recorded from the left sixth intercostal space at the point of maximal cardiac impulse. Lead II electrocardiogram is recorded simultaneously. Vertical time lines occur at 0.04 second intervals. Notice the holosystolic murmur auscultated as grade 5 (out of six).
Figure 8

Dorso-ventral thoracic radiograph of dog 5M viewed from the ventral surface. Left atrial and left ventricular enlargement are marked. Pulmonary vascular markings are within normal limits.
Figure 9

Left lateral thoracic radiograph of dog 5M taken immediately after injection of 15 ml. of radiopaque medium into the left ventricle. The left ventricle and aorta are well opacified by the radiopaque material. The left atrium is also opacified—indicating mitral regurgitation. Outlines of the mitral valve leaflets appear as dark shadows between the left atrium and left ventricle.
Figure 10

Photograph of heart removed from dog 5M. Black strings are tied to chordae tendineae of mitral leaflet. The left atrium occupies the top half of the heart and the left ventricle the bottom half. Section was made through the free-wall of the left ventricle from apex to base and extending into the left atrium. Notice the thickened mitral valve leaflets and the endocardial fibrosis appearing as white "wrinkles" in the left atrium. This endocardial fibrosis probably resulted from the regurgitant jet of blood striking the left atrial endocardium.
Figure 11
Color photograph of same view as in previous figure.
Mitra l valve leaflet of dog 5M has been reflected into the left atrium, exposing the ventricular surface and yellow vegetations.
artery immediately distal to the bifurcation. Eighteen-gauge Courand needles were introduced, percutaneously, into the left femoral artery and into the left jugular vein—both facing the heart. In some animals, either another 18 gauge Courand needle or a cardiac catheter identical to the first was inserted into the right femoral artery. The tip of the catheter was positioned in the aorta either at the origin of the iliac arteries or at the arch. These cannulae were used either for sampling blood or for registering arterial blood pressure. The Courand needle in the left jugular vein was used for injecting either the indicator or the other medicaments. Patency of all cannulae was insured by continuous irrigation with heparinized saline.

All dogs were placed lying on their right sides. Needle electrodes were placed subcutaneously from which electrocardiograms were recorded.

**Simultaneous Indicator-Dilution Curves**

Pairs of indicator-dilution curves were recorded simultaneously following injection of indicator into the jugular vein. The paired sites were:

1. Main pulmonary artery through a cardiac catheter and femoral artery through a Courand needle,
2. Main pulmonary artery through a cardiac catheter and aortic arch through a cardiac catheter, and
3. Femoral artery through a cardiac catheter and femoral artery through a Courmand needle.

The indicator was indocyanine green. Properties of this dye have been reviewed thoroughly by Fox et al. (13). Briefly, this indicator has peak absorption at 805 millimicrons, the wave length at which oxyhemoglobin and reduced hemoglobin absorb light equally. Thus, alterations in oxygen content of the blood have minimal effect on the optical density of the blood through which a monochromatic light source of 800 millimicrons is passed.

Two and one-half milligrams of indicator in one milliliter of distilled water were injected with a tuberculin syringe calibrated to inject the precise amount through an 18 gauge Courmand needle. Duration of injection was never in excess of one second and usually was less than 0.5 seconds. Subsequent injections were made after the Courmand needle was emptied of residual indicator and again filled with blood.

Sampling of systemic arterial and of pulmonary arterial blood through the cuvette densitometers was begun one to two seconds prior to injection of indicator. Constant and simultaneous withdrawal at a rate of 0.6 milliliters per second was accomplished with a Gilford Model 105-S constant flow system (14). The pump consists of two 30 milliliter syringes mounted on a double yoke driven by a constant speed motor. The ratio of sampling
rate per second to volume contained between tip of cannulae and the cuvette of the densitometer was four, a value low enough to be considered by Jose and Milnor (25) to permit recording dye-dilution curves with little significant distortion. (The volume was approximately 5 milliliters from the tip of the catheter to the cuvette and approximately 3 milliliters from the tip of the Cournand needle to the cuvette.)

Alterations in optical density of blood due to the indicator were measured in cuvette densitometers (Model No. 103-IR) manufactured by the Gilford Instrument Laboratory. The instrument consists of three basic units. The cuvette, through which blood and dye mixture is run, consists of two glass plates separated by three quarters of a millimeter distance. A beam of light, well collimated through a system of condensing lenses, is filtered to produce monochromatic light with a wave length of 805 millimicrons. A Dumont, #11430, infra-red responsive photo-multiplier was used to detect the changes in light absorption of the blood and dye mixture. The third portion of the unit consists of a power supply for the photo-tube and amplifier. A controlled circuit and correction network provide an output linear with respect to optical density. Dynamic characteristics of this densitometer have been reported previously.

Outputs from both densitometers were recorded, simultaneously, on a Sanborn, polyviso, electrocardiograph
at paper speed of either 2.5 or 5 millimeters per second. Equi-sensitive deflections were obtained for both densitometer recording systems by use of test wedges of identical optical density. A 90 per cent response of the entire system to a "square wave" bolus of dye and blood required 0.55 seconds for one densitometer and 0.48 seconds for the other.

Following inscriptions of the indicator-dilution curves, known concentrations of indicator in blood were drawn through the densitometers to provide calibrations. The concentrations were 5 and 10 milligrams per liter. The response was linear to 5 per cent.

**Experimental Groups**

Indicator-dilution curves were obtained from 20 normal dogs inscribed simultaneously from the main pulmonary artery (sampling through a cardiac catheter) and from the femoral artery (sampling through the Cournand needle). A similar technique was executed on 7 dogs with varying degrees of mitral regurgitation. These comprise the first and second groups of dogs. In a third group of dogs, indicator-dilution curves were inscribed simultaneously with sampling from the same site in the aorta—the samples being drawn through a cardiac catheter and a Cournand needle. In the fourth group of dogs, curves were inscribed as with the first two groups; however, femoral arterial blood pressures were monitored through a cannulae in the contra-lateral femoral artery. A column of fluid contained in rigid tubing
connected the Courmand needle with a Statham P23A pressure transducer, the output of which was recorded simultaneously with the indicator-dilution curves and the electrocardiogram on the Sanborn electrocardiograph. In this group of dogs the central venous volume was altered, transiently, by the administration of angiotensin, 5 micrograms administered intravenously. The indicator-dilution curves were inscribed before the administration of either isoproterenol or angiotensin and for 30 seconds immediately following the administration when a blood pressure response was observed.

Because of the availability of a Mosley autograph, strip-chart recorder with a 25 centimeter pen excursion and a frequency response of one-half cycle per second, indicator-dilution curves were inscribed from the main pulmonary artery and the femoral artery from 10 additional dogs. Since only one channel was available, a curve was inscribed from the femoral artery, then from the main pulmonary artery, and again from the femoral artery. If the curves from the femoral artery are super-imposable, it is assumed that the status of the cardio vascular system during inscription of all three curves was similar, and that the curves from the femoral and from the main pulmonary artery could have been inscribed simultaneously.

Analysis of Data

Indicator-dilution curves from the dogs in groups 1 and 2 were analyzed only for the slope of the decay in
concentration. The slope was established by constructing a tangent to the curve at a point where the decay in concentration was linear; or, if the decay was truly logarithmic and had no obvious linear decay, the tangent was constructed at a point one-fourth the distance between the peak concentration and the zero concentration line. The slope was taken as the angle formed between this tangent and the zero concentration. Hereafter, this will be called the decay slope. To minimize the admittedly rather subjective construction of this tangent, six photographic reproductions were made of each curve and tangents were constructed to each. The angles formed were averaged and this value was used as representative of the slope. The mean coefficient of variation of all sets of six reproductions for all curves analyzed was 5 per cent. Thus, the mean standard deviation of all sets of six was close to 5 per cent of the mean slope for each curve. The ratio of decay slopes for curves from the main pulmonary artery and from the femoral artery was calculated by dividing the angle formed between the tangent and zero concentration line from the main pulmonary artery curve by the corresponding angle from the femoral artery curve. Hereafter, this will be called the slope ratio.

This was the only analysis performed on curves inscribed for dogs of Groups 1, 2, 3 and 5. From dogs of Group 4, the following parameters were analyzed: cardiac output, central venous volume, mean circulation time,
arrival time, heart rate, stroke volume, and the previously mentioned ratio between decay slopes of the main pulmonary artery and of the femoral artery. Cardiac output and central venous volume were calculated according to the techniques of Stewart and Hamilton (18).

The heart rate was taken from the electrocardiogram and the stroke volume was calculated by dividing the cardiac output by heart rate. Both cardiac output and central venous volume were calculated from the femoral artery indicator-dilution curve. To express the alteration in any parameter generated by administration of angiotensin, the value obtained after the administration of the medicament was divided by the value obtained before the administration. Thus the figure expressed in the results is the per cent that the experimental value is of the control value.
CHAPTER V
RESULTS

Before simultaneous indicator-dilution curves satisfactory for analysis were obtained, many problems of instrumentation had to be overcome and many futile experiments were conducted. The following are examples of some of the problems encountered and their final solutions.

Cavitation of the tubing connecting the cannulae with the cuvettes of the densitometer occurred. This was obviated by selecting polyethylene tubing with rigid walls and internal diameter narrow enough that the volume of blood within the length of tubing was minimal (less than 0.5 milliliters) but that a flow rate of 1 milliliter per second was possible. An optimal rate of sampling through the catheter system was sought so that the sampling would be rapid enough to maintain homogeneity between red blood cells and plasma, but not so rapid that the total duration of the withdrawal could exceed 25 seconds. It was observed that, during the last five milliliters of the 30 milliliter syringes used for withdrawal on the pump, the rate of withdrawal was not constant. Thus, it was necessary for the major portion of the primary circulation of dye to have
occurred before the plunger of the syringe reached the 20 milliliter mark. Considerable time was required to adjust both densitometers and their respective read-out apparatus to equal sensitivity.

Problems existed with experimental preparation as well. Originally, a Number 8F, Cournand cardiac catheter was introduced into the main pulmonary artery; however, with the relatively rapid rate of withdrawal through the catheter, portions of the pulmonary arterial wall were aspirated into the tip and the constant withdrawal of blood was interrupted. This problem was obviated by using NIH-tip cardiac catheters which have an occluded tip but many openings spiraled on the side of the catheter near the tip. Sampling through the Cournand needles presented an even greater problem. If the flow of blood through the artery in which the Cournand needle was placed was not relatively unimpeded, marked distortion to the indicator-dilution curve recorded from that site occurred. The peak concentration was low and the slope extremely gradual. To obviate this problem, the tip of the Cournand needle was placed in the aorta through which unimpeded flow of blood and dye mixture was assured. The relatively large slope ratio observed in normal dog 18 may be attributed to stasis within the femoral artery in which the Cournand needle was placed.

The experiments reported in this paper were not conducted until the author was able to obtain three
consecutive simultaneous indicator-dilution curves in which the cardiac output calculated from the main pulmonary artery curve and from the femoral artery curve agreed to within 15 per cent and in which the femoral artery curves agreed to within 15 per cent.

Simultaneous Indicator-Dilution Curves from Normal Dogs

Simultaneous indicator-dilution curves were recorded from 20 normal dogs. Ten of these curves appear in Figure 13. The ratios of the slopes in the main pulmonary artery to those in the femoral artery (slope ratios) appear in Table 1. The mean ratio is 1.05, the standard deviation 0.05 and the range is from 1.00 to 1.16. It should be noted that the range of 1.00 to 1.09 includes 95 per cent of the dogs, and from 1.00 to 1.05 includes 65 per cent.

Indicator-Dilution Curves from Dogs with Mitral Regurgitation

Simultaneous indicator-dilution curves were recorded from seven dogs with clinical evidence of mitral regurgitation. The indicator-dilution curves from five of these animals appear in Figure 14. The mean and standard deviation are 1.36 and 0.23, respectively. The ratios appear in Table 2. The range of ratios is from 1.09 to 1.65.
Figure 13

Indicator-dilution curves from the main pulmonary and femoral arteries of 10 normal dogs. Vertical time lines occur every one second in animals 6 and 8, and occur every two seconds in animals 1, 2, 3, 4, 5, 7 and 9. Main pulmonary artery curve appears above the femoral artery curve in animals 1, 3, 5 and 8. Femoral artery curves appear above those from the main pulmonary artery in animals 2, 4, 6, 7, 9 and 10. Decay tangents, as described in the text, are constructed to the curves from animals 7 and 9. Notice the similarity in decay slopes between the two sampling sites.
Figure 14

Indicator-dilution curves from the main pulmonary and femoral arteries of 5 dogs with clinical evidence of mitral regurgitation. Main pulmonary artery curves appear above those from the femoral artery in dogs 1 and 2. Vertical time lines occur every one second in all animals. Curve inscribed from the femoral artery is inverted in animals 1 and 2. Notice the more gradual decay in concentration in curves from the femoral artery sampling site as compared with those from the main pulmonary artery sampling site.
**TABLE 1**

SLOPE RATIOS FOR NORMAL DOGS (DECAY SLOPE MPA/DECAY SLOPE PA)

<table>
<thead>
<tr>
<th></th>
<th>Slope Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>1.02</td>
</tr>
<tr>
<td>3</td>
<td>1.08</td>
</tr>
<tr>
<td>4</td>
<td>1.01</td>
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<td>6</td>
<td>1.00</td>
</tr>
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<td>7</td>
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<td>1.01</td>
</tr>
<tr>
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<td>1.06</td>
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</tr>
<tr>
<td>19</td>
<td>1.02</td>
</tr>
<tr>
<td>20</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mean: 1.04  
S.D.: 0.05

**TABLE 2**

SLOPE RATIOS FOR DOGS WITH MITRAL REGURGITATION

<table>
<thead>
<tr>
<th></th>
<th>Slope Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1M</td>
<td>1.09</td>
</tr>
<tr>
<td>2M</td>
<td>1.11</td>
</tr>
<tr>
<td>3M</td>
<td>1.47</td>
</tr>
<tr>
<td>4M</td>
<td>1.18</td>
</tr>
<tr>
<td>5M</td>
<td>1.65</td>
</tr>
<tr>
<td>6M</td>
<td>1.49</td>
</tr>
<tr>
<td>7M</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Mean: 1.36  
S.D.: 0.23
The mitral valve from dog 1M appeared only slightly thickened. The valve from dog 5M was damaged most severely. Dogs 3M and 7M had mitral valves damaged severely, while valves from dogs 2M, 4M and 6M were intermediate. The assessment of severity of valve damage is admittedly subjective and was made after analysis of the indicator-dilution curves; however, as shown in Figure 15, good correlation between slope ratios and appearance of the valve was present.

**Simultaneous Indicator-Dilution Curves Before and After Angiotensin II**

Simultaneous indicator-dilution curves were recorded before and after the administration of angiotensin II to seven normal dogs. An example of the curves registered appears in Figure 16. The slope ratios and the ratios of the slope ratios recorded before and after angiotensin appear in Table 3. The mean slope ratio before angiotensin is 1.05. The standard deviation is 0.09, and the range is between 1.00 and 1.23. The large slope ratio in animal 5 resulted, probably, from poor circulation through the vessel from which the arterial sample was taken. Even so, the data should be applicable, since the point of interest is in alteration between pre and post angiotensin periods. The mean of the slope ratios after angiotensin is 1.16, with a standard deviation of 0.11 and a range between 1.03 and 1.34. The mean ratio of the slope ratios before
Figure 15

Slope ratios (ordinate) plotted against severity of valve damage as estimated at post mortem (abscissa). Se = severe, MS = moderately severe, M = moderate, Sl = slight.
Figure 16

Indicator-dilution curves from the main pulmonary artery (top) and from the femoral artery (bottom) taken before (A) the administration of angiotensin and after (B). Blood pressure recording appears at the top. One second time pips occur at the bottom.
## TABLE 3

SLOPE RATIOS FROM NORMAL DOGS BEFORE AND AFTER ANGIOTENSIN II

<table>
<thead>
<tr>
<th>Animal</th>
<th>Before</th>
<th>After</th>
<th>Ratio After Ratio Before</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.06</td>
<td>1.03</td>
<td>0.97</td>
</tr>
<tr>
<td>2.</td>
<td>1.16</td>
<td>1.14</td>
<td>0.98</td>
</tr>
<tr>
<td>3.</td>
<td>1.02</td>
<td>1.20</td>
<td>1.18</td>
</tr>
<tr>
<td>4.</td>
<td>1.00</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>5.</td>
<td>1.23</td>
<td>1.34</td>
<td>1.09</td>
</tr>
<tr>
<td>6.</td>
<td>1.00</td>
<td>1.19</td>
<td>1.19</td>
</tr>
<tr>
<td>7.</td>
<td>1.00</td>
<td>1.04</td>
<td>1.04</td>
</tr>
<tr>
<td>Mean</td>
<td>1.07</td>
<td>1.16</td>
<td>1.09</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.09</td>
<td>0.11</td>
<td>0.10</td>
</tr>
</tbody>
</table>
to those after is 1.09, with a standard deviation of 0.10 and a range of 0.97 and 1.20.

Although in all animals the central venous volume was increased by angiotensin, in three animals the slope ratios remained essentially unchanged (animals 1, 2 and 7), while in four animals (3, 4, 5 and 6) the slope ratios increased and mimicked the slope ratio observed in mitral regurgitation. In only two cases (3 and 6) where the control slope ratio could be considered within limits for normal animals did the slope ratio become abnormal after angiotensin.

The effects of angiotensin II on slope ratio, cardiac output and central venous volume may be observed in Table 4.

From the mean values before and after angiotensin, one observes a consistent but slight decrease (20 per cent) in cardiac output and a consistent but slight (10 per cent) increase in central blood volume. The ratio of mean slope ratio after angiotensin to that before the drug was administered is 1.10.

In plots of per cent change in central venous volume against both per cent change in slope ratio and per cent change in cardiac output (Figure 17) little correlation between the parameters exists. In a plot of per cent change in slope ratio against per cent change in cardiac output, also little correlation exists (Figure 18).
VALUES FOR SLOPE RATIO (S.R.), CARDIAC OUTPUT (C.O.) AND CENTRAL VENOUS VOLUME (CVV)
FOR SEVEN DOGS BEFORE AND AFTER ANGIOTENSIN-II

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control S.R.</th>
<th>Control C.O.</th>
<th>Control CVV</th>
<th>after Angiotensin-II S.R.</th>
<th>after Angiotensin-II C.O.</th>
<th>after Angiotensin-II CVV</th>
<th>after Control S.R.</th>
<th>after Control C.O.</th>
<th>after Control CVV</th>
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<tr>
<td>1A</td>
<td>1.06</td>
<td>2.3</td>
<td>306</td>
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<td>1.8</td>
<td>330</td>
<td>0.97</td>
<td>0.78</td>
<td>1.08</td>
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<tr>
<td>2A</td>
<td>1.16</td>
<td>2.0</td>
<td>363</td>
<td>1.14</td>
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<td>420</td>
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<td>0.90</td>
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<tr>
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<td>1.02</td>
<td>2.3</td>
<td>365</td>
<td>1.20</td>
<td>1.6</td>
<td>391</td>
<td>1.18</td>
<td>0.70</td>
<td>1.07</td>
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<tr>
<td>4A</td>
<td>1.00</td>
<td>2.2</td>
<td>384</td>
<td>1.20</td>
<td>1.9</td>
<td>403</td>
<td>1.20</td>
<td>0.86</td>
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<td>5A</td>
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<td>546</td>
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<td>2.5</td>
<td>460</td>
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<td>612</td>
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<td>0.88</td>
<td>1.33</td>
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<tr>
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<td>2.5</td>
<td>460</td>
<td>1.04</td>
<td>2.5</td>
<td>560</td>
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<td>1.00</td>
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<tr>
<td>Mean</td>
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<td>2.3</td>
<td>412</td>
<td>1.16</td>
<td>2.0</td>
<td>469</td>
<td>1.08</td>
<td>0.87</td>
<td>1.14</td>
</tr>
</tbody>
</table>
Figure 17

Plot of per cent change in central venous volume (abscissa) against per cent change in slope ratio (top line) and per cent change in cardiac output (bottom line).
Figure 18

Plot of per cent change in slope volume (abscissa) against per cent change in cardiac output (ordinate).
Simultaneous Indicator-Dilution Curves from the Same Site in the Vascular System, only Sampled through Different Catheters

Simultaneous indicator-dilution curves inscribed with sampling from the same site in the descending aorta through a cardiac catheter and through a Courand needle were recorded from ten dogs. The slope ratios between catheter and needle samples are shown in Table 5. Illustrations of the curves appear in Figure 19. The mean slope ratio is 1.01 with a range of from 1.00 to 1.03. Because of the narrow range, estimations of standard deviation would be of no added meaning.

Objections have been made with respect to comparing two indicator-dilution curves inscribed with sampling through catheter systems of different volumes and/or lengths. It is obvious from the data in Table 4 that the difference in slopes between the two sampling systems (a mean slope ratio of 1.01) is negligible with respect to the differences in slopes between indicator-dilution curves from the femoral and main pulmonary arteries (a mean slope ratio for normal dogs is 1.04). Although this statement may be made with great certainty for the catheters used in this study, the statement is most assuredly false if catheters of greatly varied volume, or through which blood is sampled slowly, are used.
TABLE 5

Slope Ratios from Seven Animals in Which Sampling for Both Curves Was from a Common Site in the Aorta. One Sample Was Drawn Through a Cardiac Catheter (Volume 2 ml.), the Other Through a Courmand Needle (Volume 1 ml.)

<table>
<thead>
<tr>
<th>Needle</th>
<th>Catheter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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</tr>
<tr>
<td>2.</td>
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</tr>
<tr>
<td>3.</td>
<td>1.02</td>
</tr>
<tr>
<td>4.</td>
<td>1.02</td>
</tr>
<tr>
<td>5.</td>
<td>1.07</td>
</tr>
<tr>
<td>6.</td>
<td>1.03</td>
</tr>
<tr>
<td>7.</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>1.03</td>
</tr>
</tbody>
</table>
Figure 19

Simultaneous indicator-dilution curves from the same arterial site in nine normal dogs. Top curves are inscribed with sampling through a Courmand needle, and bottom curves are inscribed with sampling through a 50 cm. cardiac catheter. Vertical time lines occur every one second in animals 1 through 6, and every two seconds in animals 7 through 9.
CHAPTER VI

DISCUSSION

Results of this study provoke at least three questions relevant to the clinical application of the simultaneous indicator-dilution technique for quantitating mitral regurgitation. (1) Are the differences in slope ratios between dogs with mitral insufficiency and normal dogs statistically significant? (2) If this is so, is it true that the more severe the regurgitation, the larger will be the slope ratio? (3) If answers to the former questions are true, does regurgitation per se alter the slope ratio; or, is a sequel, which may be proportional to the degree of regurgitation, responsible for the alteration?

When differences between slope ratios of normal dogs and dogs with mitral regurgitation were submitted to the student "t" test to determine the probability that their means (1.04 and 1.36) differ by chance, the probability was less than one in one hundred--obviously statistically significant. However, the chi square statistic was calculated for both groups (12.86 with 5 degrees of freedom for normals and 7.04 with 3 degrees of freedom for dogs with regurgitation), and neither was found to be composed of
individuals which were distributed normally (Gaussian distribution). This reduces the value of a statistical analysis, since such a procedure requires Gaussian distribution. Absence of normal distribution may occur possibly because the parameters analyzed were in fact not distributed normally, or because the sample size was inadequate. Because of the relatively small coefficient of variation of the slope ratios from normal dogs, it is highly unlikely that the true mean of all normal dogs would vary greatly from the mean of this group.

Furthermore, it is unlikely, in light of the large mean and standard deviation for dogs with mitral regurgitation, that the mean of all dogs with mitral regurgitation of any severity would approach even three standard deviations of the mean for the group of normals.

This introduces the second question: Does the degree of mitral regurgitation parallel the magnitude of the slope ratio—or *vice versa*? This would be amenable to solution if one could estimate the severity of regurgitation. Unfortunately, no positive measure exists. The "final word" seems to lie in the finger of the surgeon who, at the time of surgery, introduces it into the left atrium and "quantitates" the magnitude of the regurgitant jet. Obviously this method, at best, is subjective—dependent upon the velocity with which the blood strikes the finger as well as the surgeon's sensory threshold for the day. One is
unable to correlate, objectively, the degree of regurgitation with the slope ratio in this study. One may say, however, that those hearts with mitral valves that appear most severely gnarled and which had the greatest endocardial fibrosis within the left atrium, did have the highest slope ratio. Dogs 1 and 2, with comparatively few valvular changes, had the slope ratio closest to the normal dogs.

Again, it must be emphasized that none of these morphological observations need necessarily parallel the physiological regurgitation. Experiments utilizing a prosthetic mitral valve, which may be made more or less "incompetent," have been designed and shall be executed in the near future.

Information obtained from studies with angiotensin II assists in answering the question of whether the increase in slope ratios observed in dogs with mitral regurgitation may be a consequence of added mixing of indicator and blood in the region of the mitral valve, or may be a sequel to inadequate transfer of blood from the pulmonary circuit to the systemic arteries and increased central blood volume.

Emanuel and Newman (12) present overwhelming evidence to suggest that the single greatest effector of the decay in the indicator-dilution curve is the largest volume of a series of volumes interposed between sites of injection and sampling. This volume, they suggest, is the volume within the lungs when indicator is injected into the jugular vein
and sampled from an artery. Under conditions of mitral regurgitation, they assert that the decreased rate of decay results from an increased volume of blood contained within the pulmonary vessels and/or left atrium. This accounts for the major alterations observed in indicator-dilution curves from subjects with mitral regurgitation; therefore, a factor which might increase the diluting volume in the central venous compartment might simulate mitral regurgitation in the indicator-dilution curve.

If an increase in central venous volume were to alter the slope ratio in normal dogs, to simulate the slope ratio in dogs with mitral regurgitation, the technique proposed in this thesis would be useless—unless the increase in central volume paralleled the degree of regurgitation. In the case where the degree of increase in central venous volume paralleled the degree of regurgitation, and if the technique proposed were sensitive to increments in central venous volume, then the technique would also measure the degree of regurgitation—"Two quantities proportional to the same quantity are proportional to each other."

It has already been established that regurgitation, alone, is not the only factor which alters the left atrial volume. Compliance of the left atrial-pulmonary venous compartment is equally important. Indeed, if the increase in central venous volume does parallel the degree of
regurgitation, one might assess the severity of regurgitation from radiographs of the thorax. This has been demonstrated to be unfeasible in clinical practice.

Comparing the means of the slope ratios from normal dogs both before and after the administration of angiotensin II, it is apparent that alteration in the central venous volume induced by angiotensin did not affect the slope ratio nearly so severely as did mitral regurgitation. Angiotensin was found to be the drug which altered central blood volume more than any other physiologic or pharmacologic maneuver—greater than negative pressure breathing, compression of the abdomen, or isoproterenol.

It must be emphasized that this study is inadequate to exclude the possibility that in normal dogs some combination of central venous volume, cardiac output or altered flow-volume relations within the vascular system may simulate the slope ratios observed in animals with regurgitation.

A question of major importance with respect to the theory of indicator-dilution curves is raised by the similarity in decay slopes of curves inscribed from the main pulmonary and femoral arteries. The assertion was made that that indicator-dilution curve was a function of total flow and dilution volume between points of sampling and injection (including all points temporally equidistant to those points) as well as any factors interposed between which
might further disperse the particles of indicator. If the assertion is correct, the slope of the indicator-dilution from the femoral artery would be, at its greatest, more gradual than the slope from the main pulmonary artery, and the peak concentration from the femoral artery would be lower than that in the main pulmonary artery. This would be the case since the volume of blood available for dilution of indicator is greater between the injection point and the point of sampling in the femoral artery than the volume between the point of injection and point of sampling in the main pulmonary artery.

The only factor which could allow the curve from the femoral artery to attain a higher peak concentration and a more rapid decay slope would be if some peculiar order of blood flow would concentrate the particles of indicator at some point in the circulation between the main pulmonary and femoral arteries. Such a factor would, indeed, be "peculiar," and is certainly difficult to imagine. Certain congenital anomalies of the heart and great vessels could exist which might permit venous blood to first traverse the systemic arterial tree and then pass to that of the pulmonary system. Animals with such lesions would most assuredly be detected by clinical examination.

Why, in most instances of simultaneous indicator-dilution curves recorded from the main pulmonary and femoral arteries of normal dogs, the decay slopes differ
by so little (a mean ratio of 1.04:1.00) suggests that the largest blood volume available for dilution exists in the venous compartment between the site of injection and point of sampling in the main pulmonary artery; and, that any volumes of the cardiovascular system more distal from the point of injection than is the main pulmonary artery contribute little to the added dispersion of indicator particles. This is contrary to information proposed in the reports from Lacy and Newman (12), who state that the pulmonary blood volume appears to be the critical volume in determining the final profile to which the indicator particles are dispersed. Their injections of indicator, however, were made into the main pulmonary artery; therefore, they could not detect the effects of venous blood volume on the dilution of indicator. They present powerful evidence that the compartment of greatest dilution volume, between points of injection and sampling, determines this final dispersion profile.

Evidence exists that the compartment of the cardiovascular system which contains the greatest anatomical volume of blood is the venous compartment. Since this venous compartment does lie within the boundary set by points of injection and sampling of the indicator, it is likely that this volume does, in fact, fix the final dispersion profile of the indicator particles. Thus, although the volumes of blood in the pulmonary capillaries,
left heart and systemic arterial tree do further disperse the indicator particles after they traverse the sampling point in the main pulmonary artery, the degree of dispersion is minimal—at least in normal animals—with regard to the degree of dispersion generated by the venous compartment.

On the other hand, when the shuffling back and forth of blood across the mitral valve is interposed between the two sampling sites, the particles of indicator are dispersed more completely within the diluting blood. This accounts for the greater discrepancy of slopes between the main pulmonary artery and femoral artery indicator-dilution curves. It may be proposed, therefore, that mitral valvular regurgitation—or any other cardiac valvular regurgitation—causes a greater degree of indicator particle dispersion than would even the interposition of a relatively great volume of blood between the sampling sites.
CHAPTER VII

CONCLUSIONS

1. Simultaneous indicator-dilution curves inscribed with sampling from the main pulmonary and femoral arteries may be recorded satisfactorily on dogs.

2. Dogs with mitral regurgitation may be separated from either normal dogs or normal dogs with an augmented central venous volume by the slope ratios between indicator-dilution curves from the two sampling sites.

3. Sampling of blood and indicator mixture through an 8F, 50 centimeter NIH-tip cardiac catheter and through an 18 gauge Courmand needle, both with a flow rate of 0.6 milliliters per second, permitted inscription of indicator-dilution curves nearly identical in configuration and timing.

4. Although too few animals with spontaneously occurring mitral regurgitation were studied with this technique to permit statistical correlation, a good correlation does appear to exist between the magnitude of the slope ratio and the severity of the mitral valve lesion at post mortem.
5. More animal experiments need to be performed to evaluate, more precisely, the factors which generate the decay slope of the indicator-dilution curves from dogs with mitral regurgitation. From further studies on dogs with spontaneous mitral regurgitation and on dogs with induced regurgitation of varying degrees, it may be possible to state more definitively if the simultaneous indicator-dilution method may be used to quantitate the degree of regurgitation.
CHAPTER VIII

SUMMARY

Indicator-dilution curves were recorded, simultaneously, from sampling sites in the main pulmonary and femoral arteries, following the injection of indocyanine green dye into the external jugular vein. Dogs were divided into three groups: 20 normal, 7 with clinical evidence of mitral regurgitation, and 7 both before and after the administration of 5 uG. angiotensin II. In an additional 10 dogs, simultaneous indicator-dilution curves were recorded from common sampling sites in the aorta, but through a cardiac catheter (used to sample from the pulmonary artery) and through a Courmand needle (from which samples were obtained from the femoral artery).

Decay slopes were calculated for all dilution curves. This was done by constructing a tangent to the point on the indicator-dilution curve where the decay in concentration was linear. This point was located, usually, within the first one-quarter of the peak concentration. A decay slope was recorded as the angle formed between the tangent and the zero concentration line. Slope ratios between curves from the main pulmonary and femoral arteries
were calculated by dividing the slope of the main pulmonary artery curve by the slope of the femoral artery curve. This quotient was always greater than unity. The larger the quotient, the greater was considered the difference in the decay slopes between the two curves. Slope ratios were also calculated for indicator-dilution curves recorded from the same sampling sites through different cannulae. The change in slope ratios induced by increasing central venous volume with angiotensin II was determined.

Mean slope ratios were: for normal dogs, 1.04; for dogs with mitral insufficiency, 1.36; for dogs after the administration of angiotensin II, 1.16. Mean slope ratio comparing curves from the same sampling site but inscribed after sampling through varying volume catheters was 1.03.

Conclusions from the data were: (1) simultaneous indicator-dilution curves could be recorded with sampling from the main pulmonary and femoral arteries; (2) differences in volumes between the two types of catheters affected the indicator-dilution curves insignificantly; (3) mitral regurgitation increased the slope ratio by a value greater than that induced by simple increase in central venous volume; (4) in general, the greater the slope ratio, the more severely damaged were the leaflets of the mitral valve; (5) more animals need to be studied to evaluate this method more definitively as a technique for quantitating the degree of regurgitation.
It was postulated that, possibly, some conditions of central venous volume and cardiac output may permit the slope ratios from normal dogs as calculated in this study to mimic the slope ratios of dogs with mitral regurgitation; however, on the basis of this study, mitral regurgitation is amenable to diagnosis by this method. It is suggested that a significant degree of added mixing of indicator particles and blood is afforded by the shuffling back and forth of blood at the mitral orifice.
REFERENCES


AUTOBIOGRAPHY

I, Robert Louis Hamlin, was born March 18, 1933, in Cleveland, Ohio. My parents are Walter E. and Sara E. Hamlin. I have two brothers. Primary and secondary education were taken in the Public School System of Cleveland Heights, Ohio. I attended Western Reserve University during 1951, and transferred to The Ohio State University where I have studied until the present time. I obtained the degrees Bachelor of Science in 1956, Doctor of Veterinary Medicine in 1958, and Master of Science in 1960. I married Beverly G. Coil in 1961. I have been a Research Fellow of the Central Ohio Heart Association and of the National Institutes of Health. I am an instructor in the Department of Veterinary Physiology and Pharmacology at The Ohio State University. My major field of interest is comparative physiology, with emphasis on comparative cardiology.