VALIDATION OF AN ALGORITHM TO ESTIMATE VASCULAR RESISTANCE AND COMPLIANCE IN A PERFUSED RAT HIND LIMB PREPARATION

A Dissertation submitted in partial fulfilment of the requirement for the Degree of Doctor of Medicine in Physiology (Branch – V) Of The Tamilnadu Dr. M.G.R Medical University, Chennai -600 032

By

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This is to certify that the thesis entitled "**Validation of an algorithm to estimate vascular resistance and compliance in a perfused rat hind limb preparation**" is a bonafide, original work carried out by Dr. Benjamin Jebaraj D, in partial fulfilment of the rules and regulations for the M.D – Branch V Physiology examination of the Tamilnadu Dr. M.G.R. Medical University, Chennai to be held in April- 2016.

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DECLARATION

I hereby declare that the investigations that form the subject matter for the thesis entitled "**Validation of an algorithm to estimate vascular resistance and compliance in a perfused rat hind limb preparation**" were carried out by me during my term as a post graduate student in the Department of Physiology, Christian Medical College, Vellore. This thesis has not been submitted in part or full to any other university.

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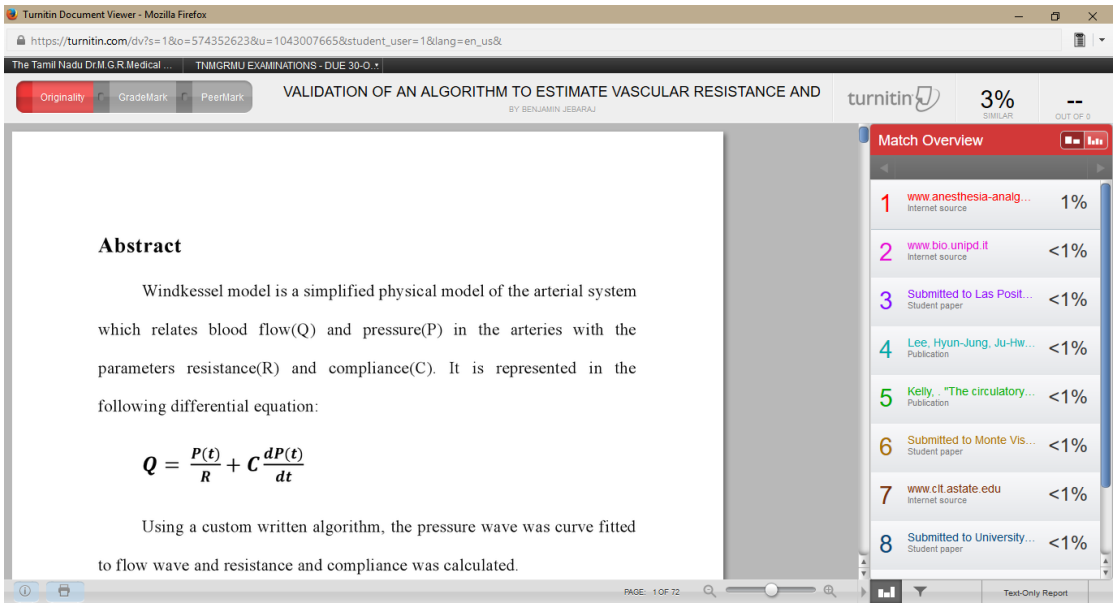
Abstract

Aim

Materials and methods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Abstract
Windkessel model is a simplified physical model of the arterial system which relates blood flow(Q) and pressure(P) in the arteries with the parameters resistance(R) and compliance(C). It is represented in the following differential equation:
$$Q = \frac{P(t)}{R} + C\frac{dP(t)}{dt}$$
Using a custom written algorithm, the pressure wave was curve fitted to flow wave and resistance and compliance was calculated.
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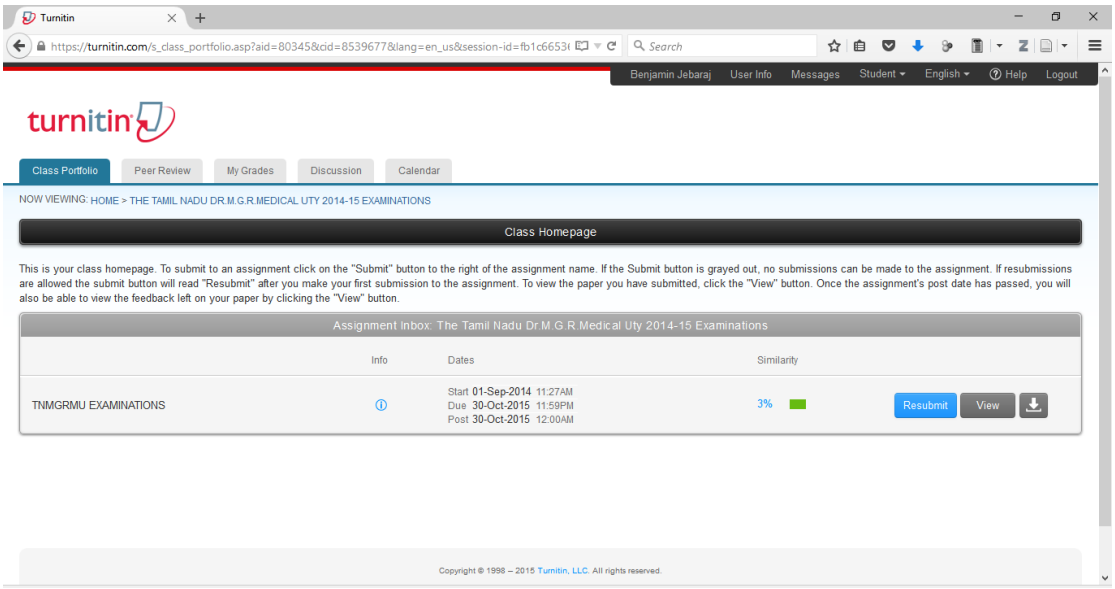
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Contents

Abstract

Windkessel model is a simplified physical model of the arterial system which relates blood flow(Q) and pressure(P) in the arteries with the parameters resistance(R) and compliance(C). It is represented in the following differential equation:

$$\boldsymbol{Q = \frac{P(t)}{R} + C\frac{dP(t)}{dt}}$$

Using a custom written algorithm, the pressure wave was curve fitted to flow wave and resistance and compliance was calculated.

Aim

Validation of an algorithm to estimate vascular resistance and compliance using windkessel model of arterial system

Materials and methods

Rats were anaesthetised, the abdominal organs were dissected and the descending aorta cannulated. The trunk was dissected below the level of diaphragm and the upper half of body removed. The hindlimb preparation was then perfused with physiological ringer

solution using an infusion pump. Pressure was recorded using a pressure transducer connected to CMCdaq data acquisition system. Since the flow rate of the infusion pump was known, the flow curve was derived. A beat to beat estimation of resistance and compliance was then obtained from the pressure recording using the estimated flow. Resistance was also calculated using the expression Mean Blood Pressure / Flow.

Results

The resistance estimated was compared to the values obtained form MBP/Flow. Pearson's correlation coefficient between the two was 0.97 ± 0.04(n=6). The Mean Absolute Percentage Error of the estimated value was 8.9 ± 3.5%.

Conclusion

A rat hindlimb preparation serves as a reliable experimental model for studying changes in vascular resistance and compliance

Introduction

The development of our knowledge of the circulation has been bedevilled by the fact that the measurement of blood flow is so complicated whereas that of blood pressure is so easy: hence the blood pressure manometer has exerted an almost hypnotic influence, though bodily organs don't need pressure but flow.

- Adolf Jarish(1928)[1]

Hypertension exerts a substantial burden on public health in India. The prevalence of hypertension in India is 28%[2]. It is an important risk factor and is the most common cause of death due to stroke and coronary heart disease[2]. The current method of diagnosis is by point measurement of blood pressure with the Korotkoff method.

The pathogenesis of primary hypertension (Essential Hypertension) is poorly understood and is compounded by multiple genetic and environmental factors. Severity of hypertension depends on the level of end organ damage and blood pressure levels per se is not an accurate predictor of severity of the disease. Treatment goals

for older individuals depends on risk factors present and there is no clear cut-off for systolic and diastolic BP targets for isolated systolic hypertension.

It is important to look for additional parameters that can complement blood pressure measurements to accurately diagnose and predict disease severity of hypertension. Blood pressure is determined by various factors like heart rate, stroke volume, peripheral resistance, blood flow and arterial compliance. Multiple factors like an increase in resistance, decrease in compliance or an increase in volume can increase blood pressure. It is not possible to diagnose the exact cause with a point measurement alone. In our opinion, ideal assessment of vascular health would involve derivation of values for resistance and capacitance from continuous arterial pressure and flow recordings done over at least an hour. In this study,

(1) we obtained

(a) continuous arterial pressure recording from a rat hind limb preparation and

(b) an estimated flow curve (where known stroke volumes from a peristaltic pump and pressure wave due to the pump output in

to a rigid tube were used to estimate real time flow for a given flow rate – this was done due to non-availability of a continuous flow meter in our lab)

(2) deployed a mathematical algorithm (Levenberg-Marquardt algorithm) to derive resistance and compliance from the two recordings mentioned above, namely arterial pressure and flow. The algorithm assumed a simple vascular system with the two element windkessel model involving a resistance and capacitance. The input for the algorithm was the flow curve and the algorithm iterated different R and C values to get an output pressure curve. The R and C values which gave a pressure curve which differed least from the recorded pressure curve were given as the actual R and C values of the system.

(3) Resistance was also calculated by conventional method as the ratio of mean blood pressure to flow. Resistance derived in this manner correlated the resistance which was derived by the algorithm.

(4) Interventions known to change resistance and compliance predictably were deployed, and the observed changes in R and C calculated with the algorithm were in agreement with what is known. The validity of the algorithm is therefore confirmed.

(5) We also observed momentary changes in compliance in response to interventions. This refutes the argument that compliance is a

constant value. This is to our knowledge the first time where compliance is shown to be a varying parameter, varying in the time range of seconds to minutes.

(6) We also observed a single instance where there is a differential response in resistance and compliance, which points towards a variation in effect of drugs at different sites of the arterial system. So studying resistance and compliance is important in understanding the physiology of the vascular system.

Review of Literature

The Cardiovascular System

Cardiovascular system includes the heart and two closed loops, the pulmonary circulation and the systemic circulation. Pulmonary circulation carries blood to the lungs, and returns oxygenated it blood to the heart. The systemic circulation carries oxygenated blood to the rest of the body and returns deoxygenated blood to the heart.

Heart

Heart is a muscular pump that beats in a pulsatile manner to pump blood through the entire circulatory system. The human heart beats at an average rate of 72 beats/min and generates enough force to pump approximately 5 litres of blood every minute. The rate at which the heart beats is the “Heart rate” and the amount of blood pumped every minute is the “Cardiac Output”.

During a beat, blood flow out of the heart occurs only during a period of contraction called the “systole” and blood flow out of the heart ceases during a period of relaxation called the “Diastole”. The

amount of blood pumped out of the heart during each systole is called the “Stroke volume”. In normal human’s stroke volume is approximately 70 ml/beat.

Vascular System

Blood from the heart reaches the aorta, and from there passes through vessels of successively smaller diameters, the arteries, arterioles and capillaries. Though the internal diameter of the vessels become low, the average cross sectional area successively becomes higher in the arteries, arterioles and capillaries because of the increase in number of branches. This reduction in cross sectional area leads to a reduction in flow velocity as blood passes through them.

Blood pressure is the lateral pressure exerted by the blood on the walls of the arteries. Blood pressure is highest in the aorta and large arteries, lower in the arterioles and capillaries and lowest in the veins. Due to the pulsatile nature of the heart, blood pressure oscillates between a high and a low. The highest point of pressure is called the systolic pressure and is 120 mmHg in humans. The lowest point of pressure is called the diastolic pressure and is about 80

mmHg in humans. The difference between the two pressures is called the pulse pressure.

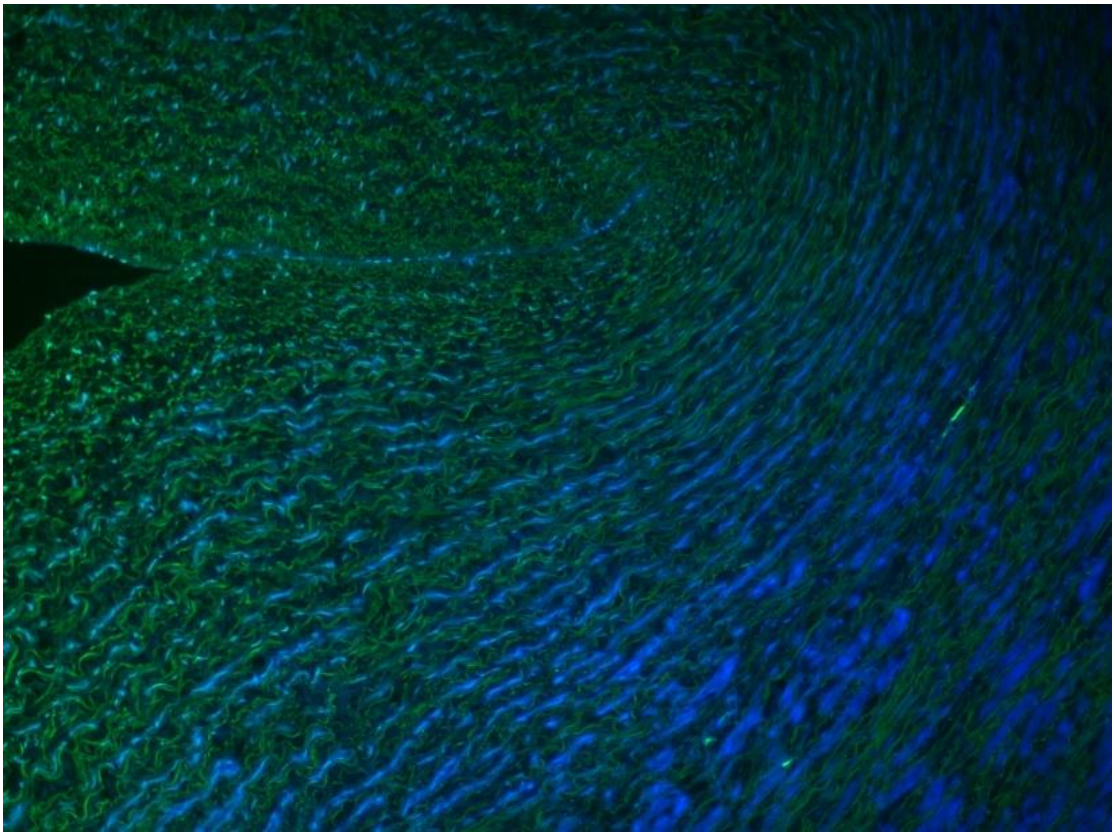
Pulse pressure is highest in the aorta and reduces as the blood flows into the small arteries. At the level of arterioles, the pulsatility ceases to exist and the pulse pressure is zero. This denotes a uniform flow of blood at the level of arterioles and capillaries.

Arteries

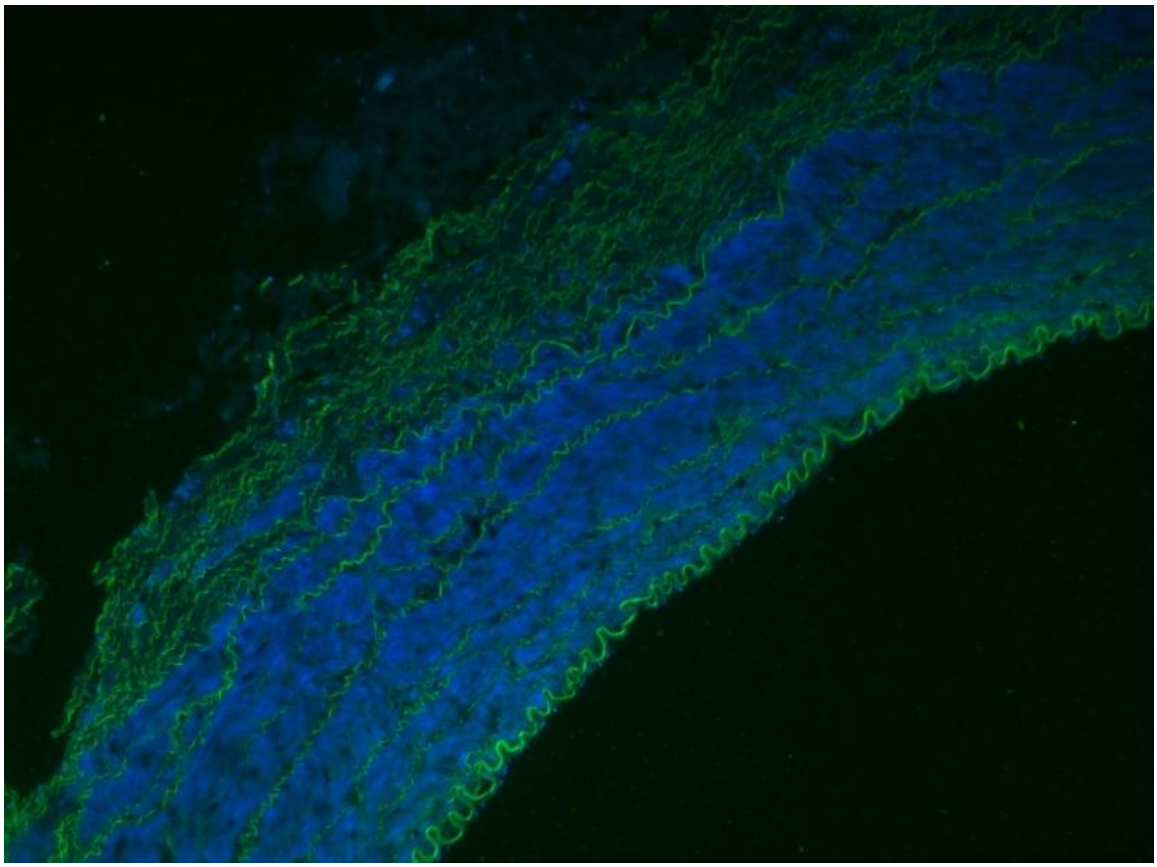
Arteries are viscoelastic structures – they distend when stress is applied and return to their normal size when stress is removed. This property is due to the presence of elastic tissues composed of elastin and collagen. The distribution of elastic tissues differs between the large arteries and the small arteries and arterioles. In large arteries the tunica media is composed of layers of elastic tissues and smooth muscle. In small arteries and arterioles, tunica media is mainly smooth muscles and the elastic tissues are confined to two layers – the internal and external elastic lamina.

Figure 1

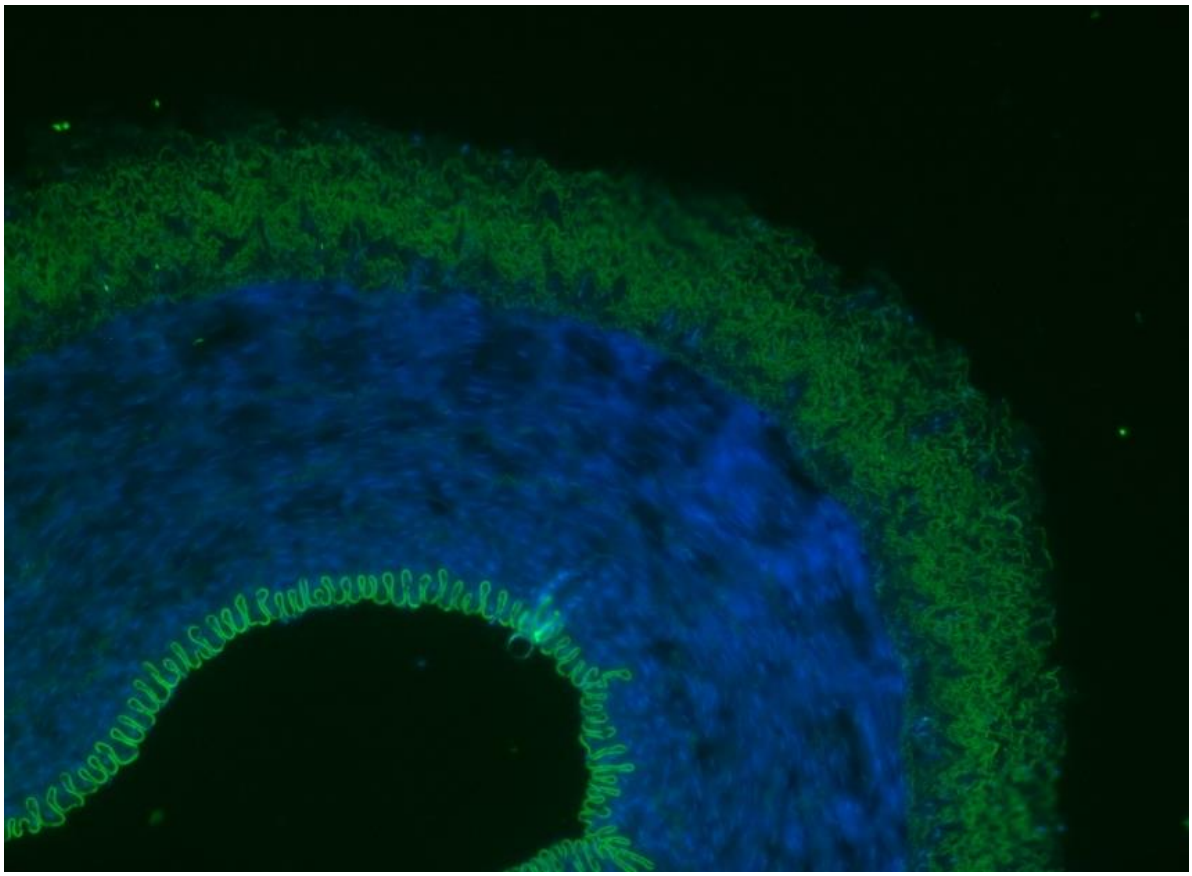
Cross section of Aorta, carotid artery and a small artery in the leg from a goat seen under fluorescence microscope. Blue colour denotes DAPI which binds to DNA and represents nucleus of smooth muscle cells. Green colour denotes auto fluorescence due to elastin and collagen and represents the elastic tissue. Images taken at Centre for Stem Cell Research, Vellore.



Aorta: Elastic tissue is present along with the smooth muscle cells, with more elastic component closer to the lumen.



Carotid artery: Smooth muscle component is higher than in the aorta. Elastic tissue is arranged in an internal elastic lamina, external elastic lamina and also interspersed in between the muscle layer.



Small artery: Elastic tissue is arranged distinctly into an internal elastic lamina and an external elastic lamina. Smooth muscle forms the major bulk of the tissue.

Determinants of arterial pressure

Arterial compliance

The aorta and large arteries, due to their content of elastic tissue, act as reservoirs of blood, so that blood flow is maintained during diastole. Their distensibility also reduces the pulsatility of flow and leads to a steady state flow in the arterioles and capillaries.

This distensibility of the arteries is described by the term Compliance. Aorta has a high compliance and can stretch easily. Arterioles has less elastic tissue and have low compliance.

Changes in compliance can be brought about in two ways

1. Changing the elasticity of vessel – this happens in case of old age. The arterial wall loses its elasticity with age and the compliance decreases.
2. changing the smooth muscle tone of the vessel – when smooth muscle contracts, it reduces the compliance of the vessel

Compliance is the change in volume for unit change in pressure. It is given by the relationship

$$Compliance\ (c) = \frac{\Delta V\ (change\ in\ volume)}{\Delta P\ (change\ in\ pressure)}$$

Change in compliance brings about changes in the pulse pressure and does not affect the mean blood pressure. If the compliance increases, the pulse pressure decreases and if the compliance decreases, the pulse pressure increases. Compliance and stroke volume are the important determinants of pulse pressure. Change in compliance also alters the contour (shape) of the pulse wave.

Arterial compliance has been identified as an important cardiovascular risk factor.[3] Studies on arterial compliance has gained importance because arterial compliance along with pressure recording can be used to derive stroke volume.

Peripheral Resistance

It is the resistance experienced by the flow of blood. It is given by the relationship

$$R = \frac{\Delta P}{Q}$$

Where ΔP is the pressure difference and Q is the flow. Pressure difference here denotes the difference in pressure between the input and output. In the context of the cardiovascular system, input pressure is the aortic pressure and the output pressure is the right atrial pressure. Since the right atrial pressure is closer to zero, the pressure difference is assumed as aortic pressure.

A more familiar form of the expression will be

$$Mean\ Blood\ Pressure = Cardiac\ output \times Total\ Peripheral\ Resistance$$

Where cardiac output is the volume of blood that flows through the circulatory system in a specific duration and Mean Blood Pressure is the average blood pressure during that duration.

The factors that influence resistance were first worked out by Poiseuille. He observed that a large drop in pressure occurs

somewhere beyond the small arteries. So he studied the flow of water through small tubes ranging from 0.03mm – 0.14 mm. He arrived at the following expression in his experiments

$$Q = \frac{kD^4(P1 - P2)}{L}$$

Where Q, (P1-P2), L and D are the volume flow, pressure drop, length of tube and diameter of tube respectively and k is a constant.

The relationship was improved by Wiedmann and Hagenbach who independently derived the following equation

$$Q = \frac{\pi r^4(P1 - P2)}{8\eta L}$$

Where r is the radius of tube and η is viscosity of fluid.

From this relationship resistance(R) can be derived as

$$R = \frac{8\eta L}{\pi r^4}$$

It can be seen that the factors affecting resistance are viscosity of liquid, length of tube and radius of tube of which radius is the most important parameter. A 2-fold change in radius brings about a 16-fold change in resistance. The radius of the aorta is 200 times the radius of the arterioles[4]. Thus the resistance offered by the change

in radius in the peripheral vasculature (arterioles) is the most important contributor and is thus termed as Peripheral Resistance.

Isolated changes in resistance will increase both the systolic and diastolic pressure and thus the mean blood pressure in increased, but the pulse pressure is not affected. Changes in resistance also do not affect the contour of the pulse wave.

Estimation of resistance and compliance

While peripheral resistance can be easily calculated by measuring pressure and cardiac output, determination of arterial compliance has been challenging. This is because calculation of compliance requires a real time recording of flow through the vessel.

There is a division among groups who study arterial compliance – those who consider compliance as a constant and those who consider compliance as a varying phenomenon. Some researchers consider compliance to be a constant value [5][6][7] but Liu et al.[8] argues that compliance is variable over time.

Estimation of compliance can help in deriving stroke volume from a blood pressure recording.

Direct estimation of stroke volume is done by one of the following methods:

Thermodilution – This is the gold standard method to measure cardiac output. A cold saline is injected into the pulmonary aorta and the change in temperature at a distal artery is measured. From the change in temperature the cardiac output is calculated.

Doppler procedure – velocity of blood can be calculated using doppler and the stroke volume can be derived from it. This is the only noninvasive method to measure flow, but requires expensive equipment and a trained sonologist.

Flowmeter – an ultrasound flowmeter is placed at the base of the aorta to measure instantaneous flow. This method is mostly used in animals for research purposes as it requires thoracotomy.

All these methods are expensive and requires experienced persons for performing the procedure.

In current clinical practice, the primary reason for estimating compliance seems to be calculation of stroke volume in real-time using compliance and a pressure recording. Compliance for this purpose is calculated by a point measurement of cardiac output. It is

then considered to be a constant value and stroke volume estimated in real time using that derived compliance value.

We believe that compliance changes moment to moment and therefore it is essential to obtain a graph of compliance in real-time. This is possible with our algorithm which assumes the two element windkessel model, if there are pressure and flow recordings in real time.

Windkessel model is the most common mathematical model used in calculations regarding the parameters that determine blood pressure.

In this study we have used windkessel model to estimate resistance and compliance beat by beat. We also show that compliance is not a constant value and changes in compliance occur in short time intervals.

Windkessel model

Mathematical models are description of systems in terms of mathematical concepts. They are frequently used in various branches of science and in engineering. Models help in learning about the various factors that affect a system and the degree to which a system is affected by a factor. Modelling helps in predicting the behavior of a system.

In biology mathematical modelling is frequently applied in fields of modelling cellular networks, protein folding and in drug development. Hodgkin-Huxley model is a mathematical model of action potentials for which Nobel Prize was awarded to Alan Hodgkin and Andrew Huxley. The Blue Brain Project[9] which aims to develop a computer model of brain has received a $1 Billion grant from the European Commission. Many models have been developed to explain the dynamics of blood in arterial circulation.

Windkessel model is a physical model of the arterial system. It was developed by Stephen Hales in 1773. The mathematical principles were laid out by Otto Frank, a German physiologist[10]. Windkessel is German for ‘air chamber’. They were used in old fire-engines to smooth out pulsatile flow. The pump ejects water out in

pulses. When flow occurs, the chamber fills up with water and when the flow stops the stored water is discharged. This ensures a smooth outflow.

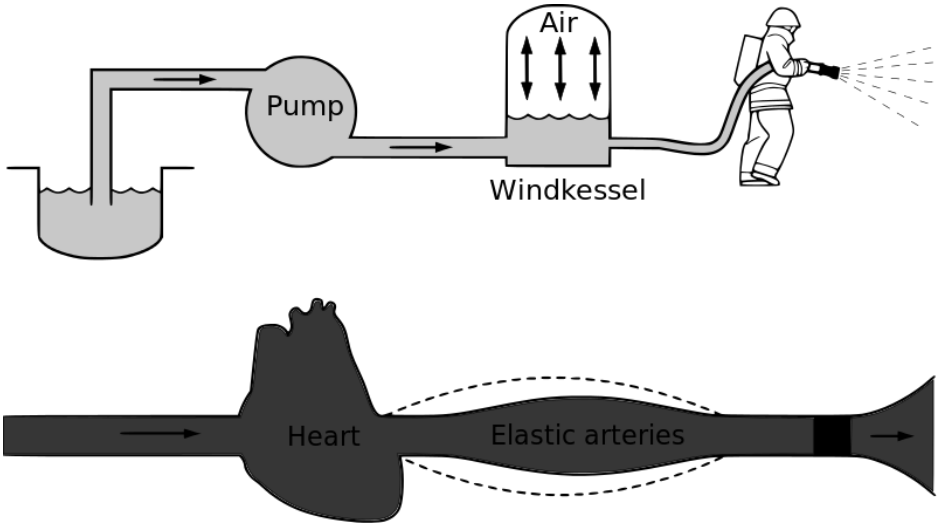


Figure 2 : windkessel mechanism. Windkessel acts as a reservoir of water to ensure smooth outflow of water. Elastic arteries act in a similar manner to reduce pulsatility of blood flow and ensure a smooth flow of blood.

In the Windkessel model of the cardiovascular system, the whole of the arterial system is considered as one single segment. During systole, the large arteries act like an elastic bag and store blood. During diastole, when there is no incoming blood from heart, the stored blood is discharged due to elastic recoil. This ensures that

there is steady flow of blood to the tissues throughout the cardiac cycle.

A two element windkessel model consists of two lumped parameters – Resistance(R) and Compliance(C). It is analogous to an electrical circuit with a resistance and capacitance in parallel.

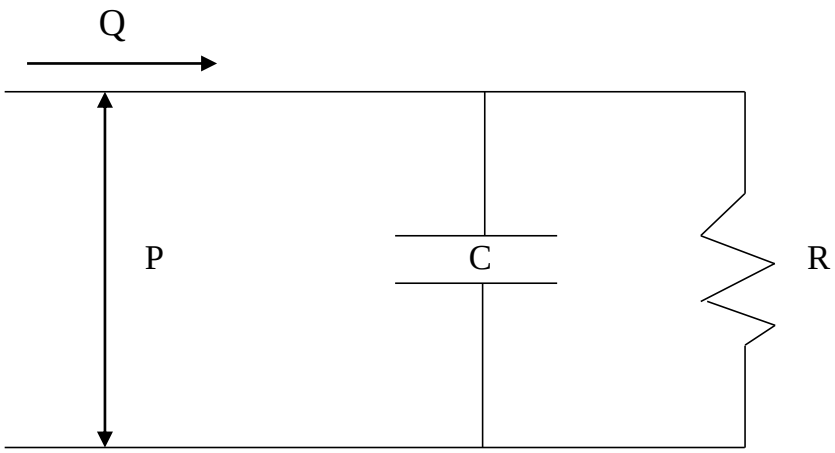


Figure 3: Electrical analogue of a 2-element windkessel model. Q is the current flow in the circuit which represents blood flow. P is the voltage difference across the ends of the circuit which represents pressure. C is capacitance and R is resistance in parallel which represents compliance and peripheral resistance respectively.

Flow across the resistance vessels is given by

$$Q_R = \frac{P}{R}$$

Where

Q_R is the rate of flow across the resistance vessels

P is the aortic pressure

R is the resistance

Flow across capacitance vessels is given by the relationships

$C = \frac{V_c}{P}$ or $V_c = C \times P$

And $Q_c = \frac{dV_c}{dt} = C\frac{dP}{dt}$

Where

Q_c is the flow rate

V_c is the flow across the vessel

C is the compliance

P is the pressure

The total flow is given by

$Q = Q_R + Q_c$ or

$$\boldsymbol{Q = \frac{P(t)}{R} + C\frac{dP(t)}{dt}}$$ ---Eq (1)

Where

Q – Flow rate

P – Pressure

R – Resistance offered to flow

C – Compliance (elasticity) of the arteries

This classical model as given by Otto Frank was later expanded to include additional parameters. The original classical model then came to be known as the two element windkessel model.

A three element windkessel model was introduced by Westerhof *et al*[11]. In this, 'Characteristic impedance' of the aorta (Z) was given as an additional parameter. Characteristic impedance denotes the resistance experienced to flow in the aorta due to the pulsatile nature of flow.

The equations for 3 element windkessel model is

$$\left(1 + \frac{Z}{R}\right) I(t) + CZ\frac{dI(t)}{dt} = \frac{P(t)}{R} + C\frac{dP(t)}{dt}$$ ---Eq (2)

A four element windkessel model accounts for the inertia of blood flow (L) and was introduced by Stergiopulos *et al*[12]. Inertia adds the effect of mass of blood on flow.

The equation for 4 element windkessel model is

$$\left(1+\frac{Z}{R}\right)I(t)+\left(CZ+\frac{L}{R}\right)\frac{dI(t)}{dt}+LC\,\frac{d^2I}{dt^2}=\frac{P(t)}{R}+C\,\frac{dP(t)}{dt}$$ ----

Eq (3)

Windkessel model is not a complete model of the cardiovascular system, since it lumps together the vessels and ignores the effect of wave reflections at the branching points. But windkessel model has been commonly applied in studies of dynamics of the cardiovascular system because of its simplicity and its relative accuracy in predicting flow or pressure. Windkessel model has been used to develop tissue engineered heart valves[13].

Three and four element Windkessel models may increase accuracy of calculations in comparison to the two element model, however they do so at the cost of simplicity of calculations. Here we have resorted to the two element WK model to describe flow and pressure changes in the aorta and have derived beat to beat resistance

and capacitance using a simple algorithm developed by the candidate submitting this thesis.

Rat hind limb preparation

The perfused rat hind limb preparation has been used extensively in exercise physiology for studies of muscle metabolism[14] and in pharmacokinetics to study tissue perfusion[15].

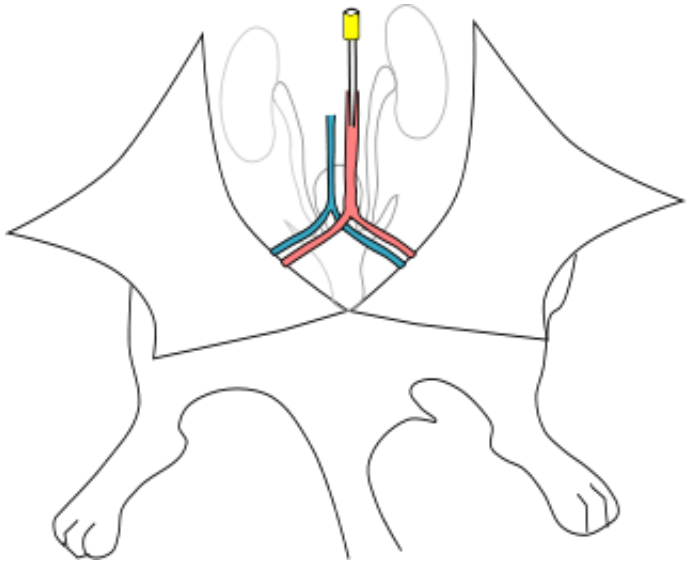


Figure 4: Model of a perfused rat hindlimb preparation. The trunk is dissected below the diaphragm and the descending aorta is cannulated.

Perfused rat hindlimb preparation was first developed by Ruderman in 1971[16]. Studies on muscle metabolism has been conducted in this model by manipulation of rate of O2 supply and effect of specific compounds on metabolic state of muscle has been studied. Perfusion of different muscles of the hindlimb using this preparation has been studied[17].

Different versions of the model are used in different settings. In a perfused rat hind limb preparation, both the hind limbs and a portion of a trunk is perfused with the help of a pump. This allows the experimenter to control the rate of perfusion and the composition of the perfusate.

This preparation was chosen for this study because it provided a subset of arterial system with intact arteries and arterioles in contrast to an isolated vessel preparation which is usually chosen to study its properties. The preparation is devoid of neural reflexes from the brain and thus the effect of interventions observed will be purely vascular changes.

Peristaltic pump

To replace the pulsatility of the heart, a peristaltic pump was used.

The peristaltic pump which was used in this study has a rotor with 6 rollers and as the rotor turns the rollers compress a rubber tube to push out the fluid.

Figure 5: The peristaltic pump used for the experiments. It has a rotor with 6 rollers. The rollers compress the rubber tube and pump the fluid through the tube.

The roller during a single rotation pushes out a constant amount of fluid. Thus it behaves like a heart with a cycle rate (analogous to heart rate) and flow rate per beat (analogous to stroke volume).

Flow rate in the peristaltic pump can be calculated as

$$Flow\ rate\ (in\ \ ml/min) = cycle\ rate(in\ \ beats/min)\ \ x\ beat\ flow\ rate(in\ \ ml/beat)$$

When the flow rate is increased in the peristaltic pump that we have used, the speed of rotation and thus the cycle rate increases but the flow rate per beat remains constant at 120 µl / beat. So

$$flow\ rate\ (in\ \ ml/min) = cycle\ rate(in\ \ beats/min)\ \ x\ 0.012\ ml/beat$$

For calculation of compliance, real-time changes in flow must be recorded which requires a flow meter. Since a flow meter was not available in our lab, flow was derived from pressure curves. For this, the output of the pump was connected to a purely resistor model, like a 26G metal needle. The metal needle dos not have any elasticity and is a purely a resistor.

In this case the relationship between real-time pressure and flow is given by

$$Q = \frac{P}{R}$$

Pressure curve during flow from peristaltic pump was obtained from the metal needle. Area under the pressure curve for each stroke of the pump was determined. This area must be proportional to the stroke volume of the pump which we determined to be a constant 120 microliters. The proportionality constant R was derived as AUC for pressure curve/120 microliters. The proportionality constant was substituted for R in the above relationship, and real-time flow curve was derived from the pressure curve.

Thus with the use of peristaltic pump, the flow is known and the windkessel model can be used to obtain a beat to beat resistance and compliance from the pressure and flow waveforms.

Curve fitting

The two element windkessel equation as given below is a non linear differential equation.

$$Q = \frac{P(t)}{R} + C\frac{dP(t)}{dt}$$

By substituting values for any of the three parameters, the value of the fourth parameter can be derived.

Among the four parameters, pressure was recorded and flow was derived as stated in the previous section. Two of the parameters R and C were unknown.

To estimate this, the flow curve was used as input in a mathematical algorithm, the Levenberg-Marquardt algorithm[18]. The algorithm employs curve fitting using Least squares method.

In ‘Least squares’ the best fit minimizes the sum of squares of the residuals (the difference between the observed value and the fitted value provided by the model).

The Levenberg–Marquardt algorithm is the standard technique used to solve ‘least squares’ problems involving non-linear equations. The algorithm was first published by Kenneth Levenberg

in 1944 and was improved in 1963 by Donald Marquardt. It is a combination of two different methods – gradient method and Gauss-Newton method

The flow curve was used as the input and the algorithm substituted parameters R and C with a starting point and a pressure curve was derived. The sum of squares of the difference between the recorded pressure curve and the derived pressure curve was calculated by the algorithm. In the next iteration, the parameters are adjusted so that the sum of squares decreases and moves towards zero. In successive iterations the parameters move closer to real values to give the least sum of squares. Finally, the parameters which gives the best fit becomes the output of the algorithm.

Validation of model

Changes in vascular resistance are brought about by contraction or relaxation of smooth muscle of the vessel walls. There are two sets of smooth muscle, longitudinal and circular smooth muscle, each affecting the nature of the vessel differently. Contraction of the longitudinal muscle increases tension of the vessel wall[19] leading to decrease in compliance. Contraction of the

circular muscle reduces the diameter of the vessel and thus increases resistance. The responsiveness of the different muscle groups tend to differ in response to different substances[20][21][22].

Current methods of measurement of compliance do not resolve beat to beat variations[23]. A one-point value of flow is obtained and the compliance is calculated. This one-point compliance is used to deduce changes in resistance.

Observations made by us had let us believe that compliance can also vary in short term. The method that we are working on will be able to measure beat to beat compliance in addition to resistance if records of BP and flow are available. Our approach to validate our method of deriving resistance and compliance from BP and flow tracings will be as follows:

Use interventions which are known to affect either one or both components of the two element model. An example of intervention which affect both is - Use of high concentration of KCl – both types of muscle (longitudinal and circular) should contract with such an intervention. By increasing the K+ concentration, KCl directly depolarizes cell membrane. This depolarization opens voltage sensitive Ca+ channels [24].

It will be seen with our analysis, if resistance and compliance change predictably with the interventions mentioned above.

Once the model is validated with the above intervention, known vasoactive drugs (adrenalin) will be added. Distribution of adrenergic receptors in circular and longitudinal muscles elsewhere are known to differ[25]. The component of change in arteriolar resistance vs arterial compliance will be studied.

Aims and Objectives

Aim:

Real time estimation of vascular resistance and compliance in mammals.

Objective:

1. Recording of arterial pressure from descending aorta which is perfused at a known flow rate in an invitro preparation of the rat where the upper half of body is removed

2. Application of different algorithms based on 2, 3 or 4 – element windkessel models and selection of the best algorithm which gives the best fit for the recorded signal.

3. use of the selected algorithm to derive beat to beat vascular resistance and compliance from the recorded signal

4. validation of the method by using interventions known to produce specific changes in vascular resistance and compliance

Materials and Methods

Experimental design

Wistar rats were anaesthetized by intraperitoneal injection of ketamine (100 mg/kg body weight). Under anaesthesia, the abdomen was opened by a transverse incision. After removal of intestines and ligation of some major branches, the descending aorta was cannulated. The animal was dissected midway through the abdomen and the upper half of the body was removed.

The cannula was connected to an infusion pump and a pressure transducer through a three-way port. A physiological salt solution was supplied through the infusion pump, which was pushing the fluid at a constant rate. Pressure was recorded using an amplifier and a data acquisition system.

After an initial period of stabilisation of 15 minutes, the intervention was administered for a period of 15 minutes, and recording was continued for another 15 minutes in the post intervention period. The recordings were acquired using CMC data acquisition software. Analysis of the pressure recordings were done using custom software

Materials

For isolated hind limb preparation

24G cannula

3-way stop cock

Scissors – curved and straight

Cotton

Artery forceps – straight and curved

Forceps

Bone cutter

Suture thread

Solutions

Ketamine – 100 mg/kg body weight

Physiological salt solution

NaCl

KCl

MgCl2

NaH2PO4

NaHPO4

NaHCO3

HEPES

Glucose

Dextran

Recording

Peristaltic pump

Pressure transducer

Pressure amplifier

CMCdaq – Data acquisition system – Hardware and software developed by Department of Bioengineering, CMC

Figure 6: CMC data acquisition system used for recording

Figure 7: Pressure transducer and amplifier used for recording. Pressure transducer is the BP transducer used in ICU for intra-arterial BP monitoring. The amplifier and CMCdaq were developed by the Department of Bioengineering, Christian Medical College

Methods

Hind limb preparation

Wistar rats of either sex weighing 230 – 300 grams were used. The rats were obtained from the Institutional animal house at Christian Medical College, Vellore.

Rat was anaesthetized with ketamine in a dose of 100mg/kg body weight. Under anaesthesia, the abdomen was opened with a transverse incision. The celiac trunk was identified and ligated. Ligatures were made above the duodenum and below the caecum. The intestines were dissected in toto and any bleeding vessels were identified and ligated. After removal of the intestines, the posterior peritoneum was opened and the descending aorta and inferior vena cava were identified. Soft tissues around the aorta were dissected out.

The descending aorta was ligated below the origin of celiac trunk. A 24G cannula was used to cannulate the descending aorta above the level of the bifurcation. After the cannula was secured in place, a bone cutter was used to dissect the spine below the level of diaphragm and the upper half of the body was removed.

Infusion setup

The hind limb preparation was perfused with a physiological salt solution using a perfusion pump. The composition of the perfusion solution is shown in Table 1

Table 1 : Composition of physiological salt solution

Salt	Concentration
NaCl	100 mM
KCl	3 mM
CaCl2	1.3 mM
NaH2PO4	2 mM
Na2HPO4	0.5 mM
MgCl2	2 mM
Hepes	10 mM
NaHCO3	25 mM
Glucose	5 mM
Dextran	3%

Dextran was added to maintain oncotic pressure of solution.

NaHCO3 and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were used a buffers. Since NaHCO3 was used as a buffer, carbogen (5%CO2 + 95%O2) was passed through the solution to maintain pH when the experiment was performed.

When 80mM KCl was administered the concentration of NaCl and KCl used were as in Table 2

Table 2: composition of high potassium solution

Salt	Concentration
NaCl	**20 mM**
KCl	**80 mM**
CaCl2	1.3 mM
NaH2PO4	2 mM
Na2HPO4	0.5 mM
MgCl2	2 mM
Hepes	10 mM
NaHCO3	25 mM
Glucose	5 mM
Dextran	3%

In a different experiment 15μM Adrenaline was added to the physiological salt solution as mentioned in Table 1.

A modified langendorf setup was used for perfusion. Two reservoirs were used, one for physiological salt solution and the other for test solution.

The reservoirs were water jacketed and the temperature of the water was maintained at 37° using a circulating water bath. The perfusate in both reservoirs were oxygenated using carbogen (95% O2 and 5%CO2) at all times. CO2 was added to enable buffering activity of NaHCO3.

The output of the reservoirs was connected using a three way to the peristaltic pump. the three way enabled switching of the solutions without disturbing the flow out of the pump.

The hindlimb preparation was connected to the output of the peristaltic pump and a pressure transducer using a three way. Pressure was recorded using an amplifier connected to the pressure transducer and CMCdaq data acquisition system. The pressure data was recorded on computer using CMCdaq software.

For recording flow waveform, the output of the pump was connected to a 26G needle to apply resistance to flow and the pressure recorded.

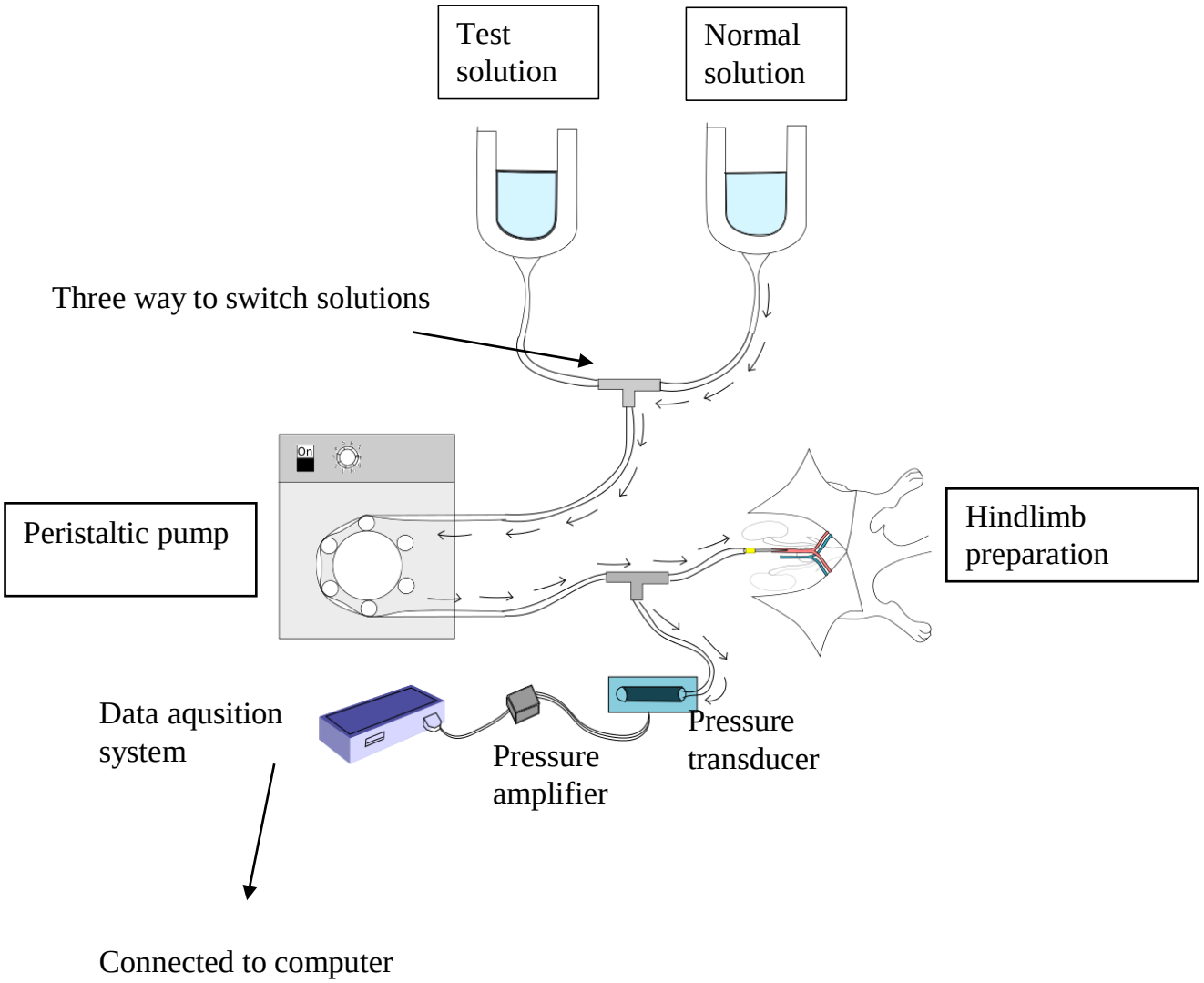


Figure 8: reference model of the infusion setup

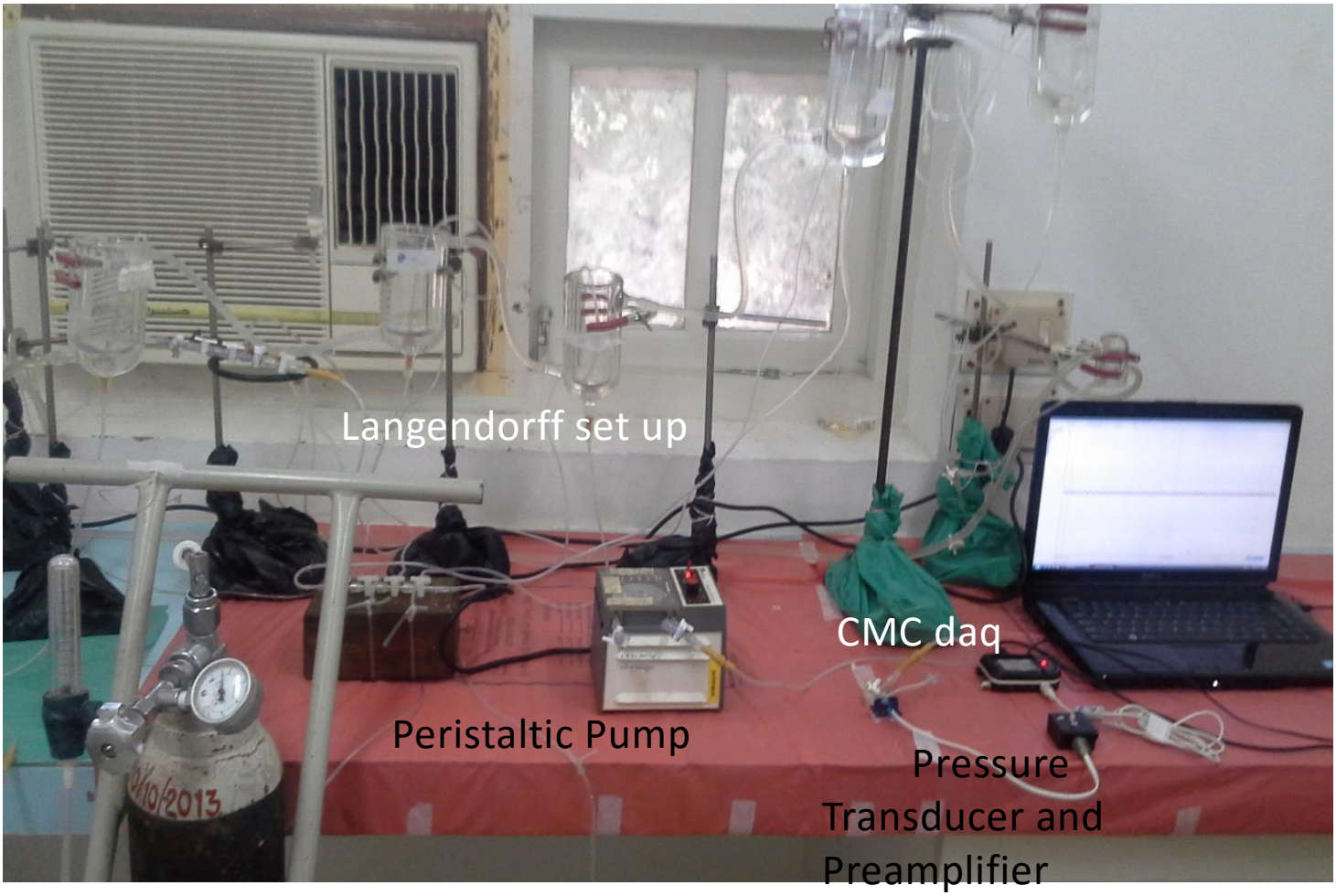


Figure 9: Animal lab where the experiments were performed

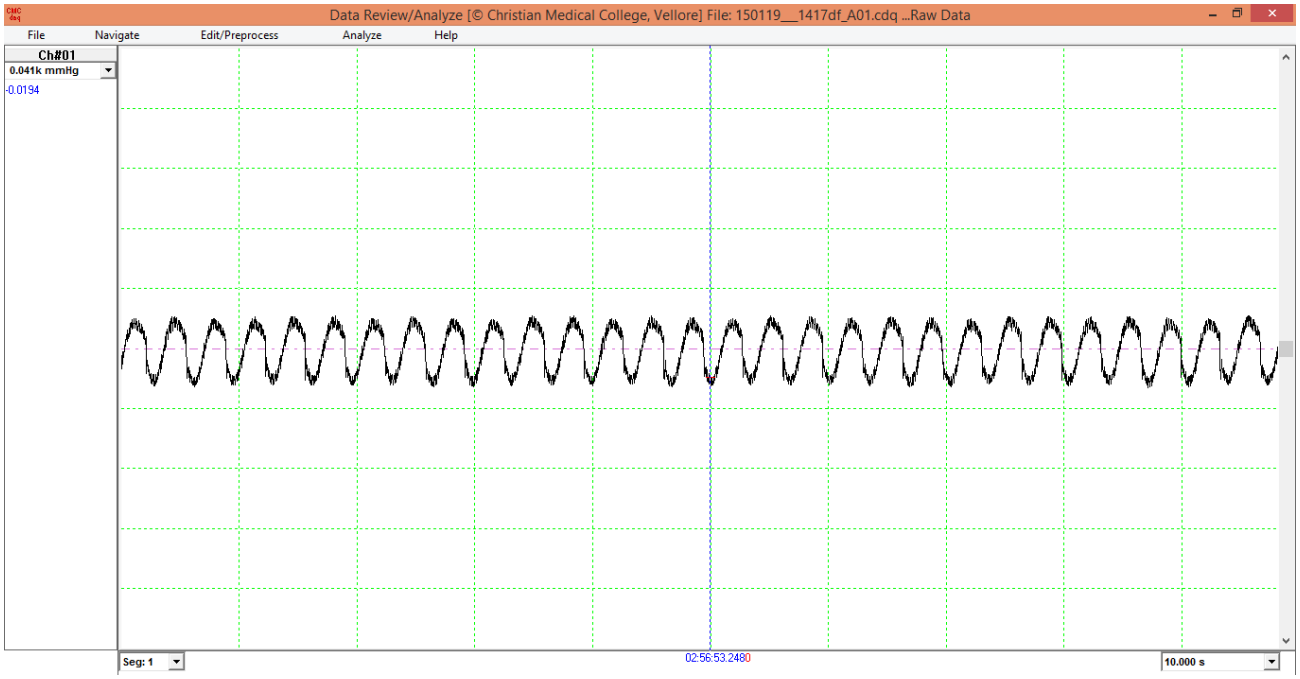


Figure 10: Recording of pressure in CMCdaq software. The pressure was recorded at a frequncy of 1000Hz. The recordings were saved in text format and analysis was done later.

Determination of flow per beat and derivation of flow waveform

The salt solution was perfused through the infusion pump and the output of the infusion pump per minute was measured using a measuring jar. The duration between two beats of different rates were determined from the pressure recording from the interval between two consecutive diastole points. The number of beats per minute was calculated as $\frac{60}{duration\ of\ each\ beat\ in\ seconds}$. The volume of flow per beat was measured as $\frac{output\ volume\ per\ minute}{no\ of\ beats\ per\ minute}$.

The measurements are given in Table 3.

Table 3

Output volume per minute (in ml/min)	Number of beats per minute	Output volume per beat (in ml/beat)
2	17.14	0.117
4	33.89	0.118
6	51.43	0.117
8	67.92	0.118
10	83.34	0.120

The flow per beat of the infusion pump was determined as 0.12 ml/beat.

To derive the waveform for flow, the output of the pump was connected to a purely resistor model, a 26G metal needle. The metal needle does not have any elasticity and is a purely a resistor.

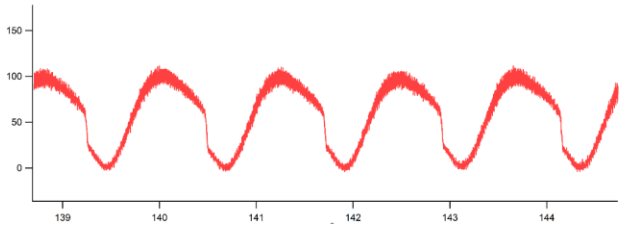
In this case the relationship between real-time pressure and flow is given by

$$Q = \frac{P}{R}$$

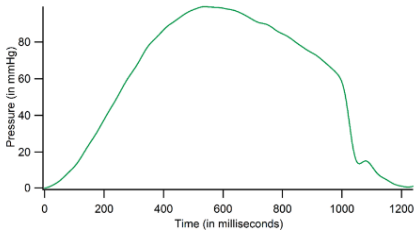
Pressure curve during flow from peristaltic pump was obtained from the metal needle. Area under the pressure curve for each stroke of the pump was determined. This area must be proportional to the stroke volume of the pump which we determined to be a constant 120 microliters. The proportionality constant R was derived as AUC for pressure curve/120 microliters. The proportionality constant was substituted for R in the above relationship, and real-time flow curve was derived from the pressure curve.

Derivation of flow waveform

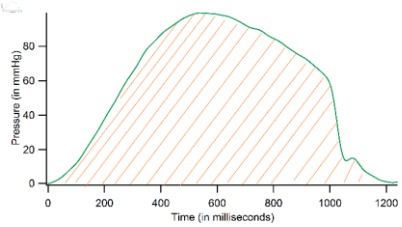
Recording of pressure with a 26G needle



Single pressure waveform

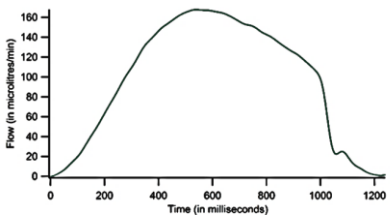


Area Under Curve (AUC) of pressure wave



Proportionality constant = AUC / Flow

Flow wave = Pressure wave / Proportionality constant



Derivation of resistance and compliance

Custom code was written using Python[26] computer language for analysis of waveforms.

The data recorded in CMCdaq was imported into the program for analysis. Data was recorded at a frequency of 1000Hz. To reduce the noise in the recorded signal, it was filtered using a 30 Hz low pass Bessel's filter.

The filtered waveform was then analysed to identify the peaks and troughs. Small segments of the recording were analysed and the maximum and minimum in that segment was identified. By iterating through the whole waveform, all the peaks and troughs were identified. The peaks correspond to systolic pressure and the troughs correspond to the diastolic pressure.

Each pressure wave was curve fitted to the flow waveform using the windkessel equation. The algorithm used for curve fitting was least squares method using Levenberg–Marquardt algorithm[27] included in the scipy module of python software. The equation used was

$$\frac{dP}{dt} = \frac{Q(t)}{C} - \frac{P(t)}{R \times C}$$

Where

$P(t)$ represents pressure at time t

$Q(t)$ represents flow rate at time t

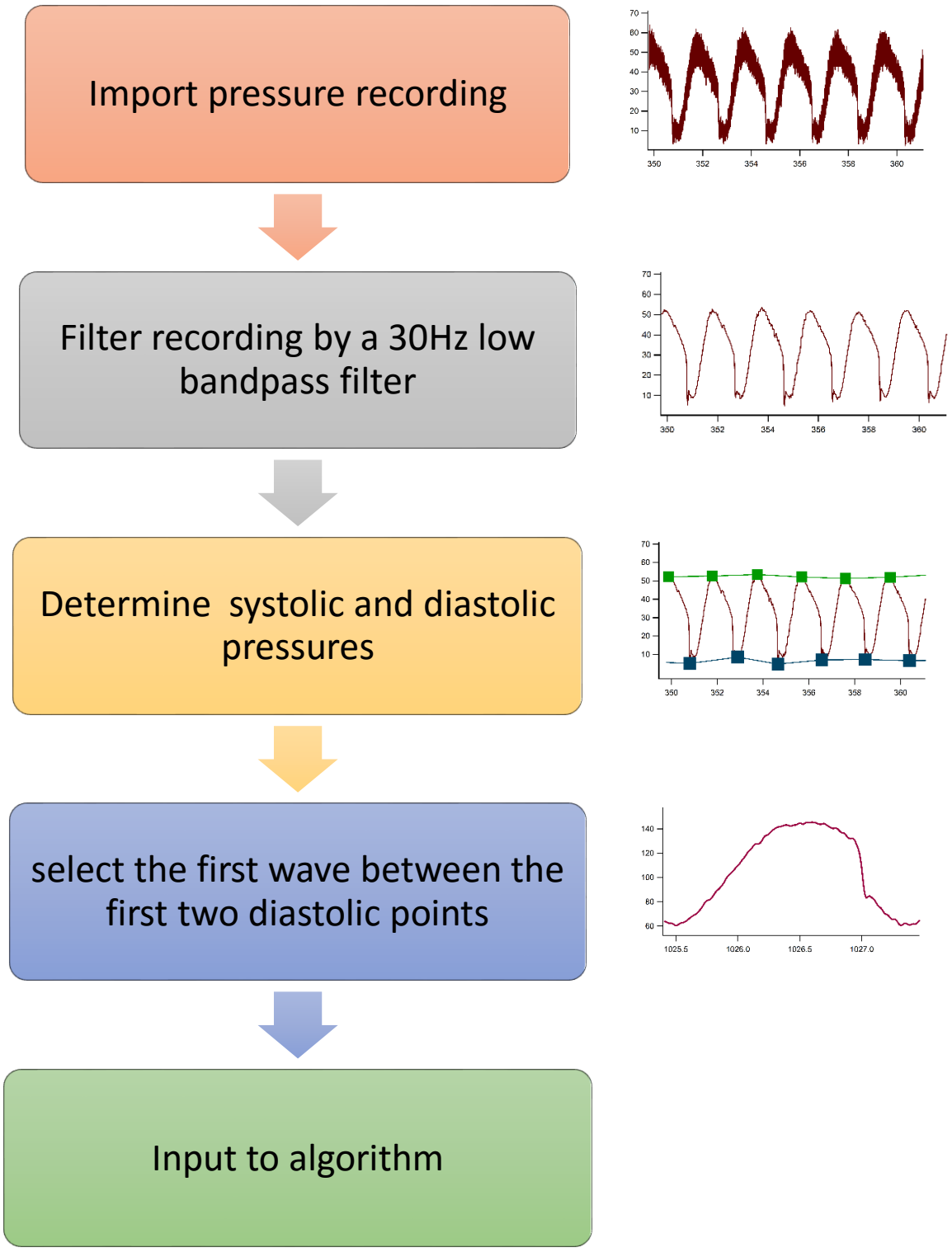
$\frac{dP}{dt}$ represents the change in pressure

R represents Resistance

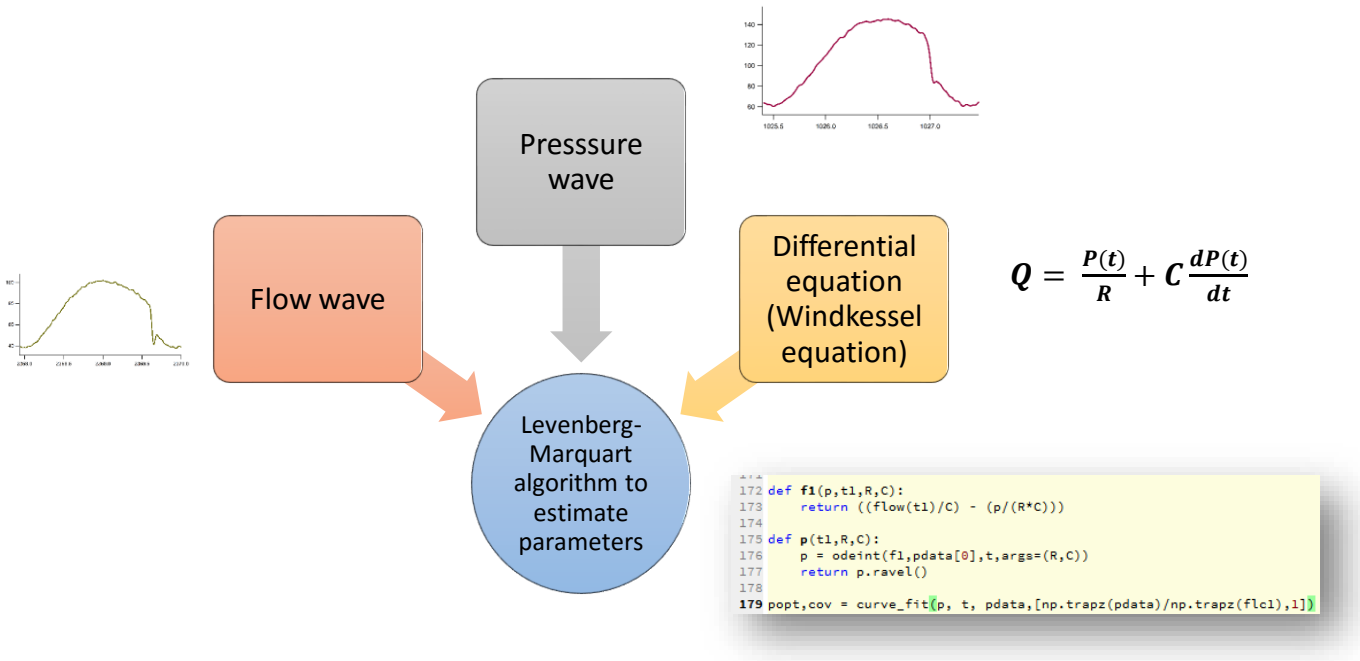
C represents Compliance

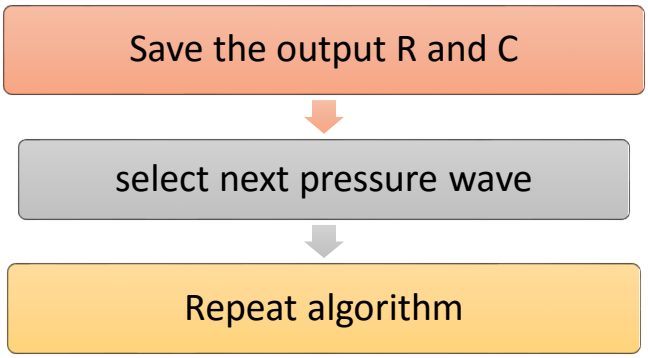
By curve fitting flow curve for pressure curve, the values of resistance and compliance were derived for each wave.

Resistance and compliance were derived from pressure waves



Flow Chart depicting the steps of analysis





Flow Chart depicting the steps of analysis (continued)

The figure below shows one of the pressure curves and the fitted curve. The fitted curve is derived from the equation using the flow, and the parameters derived by the least squares method.

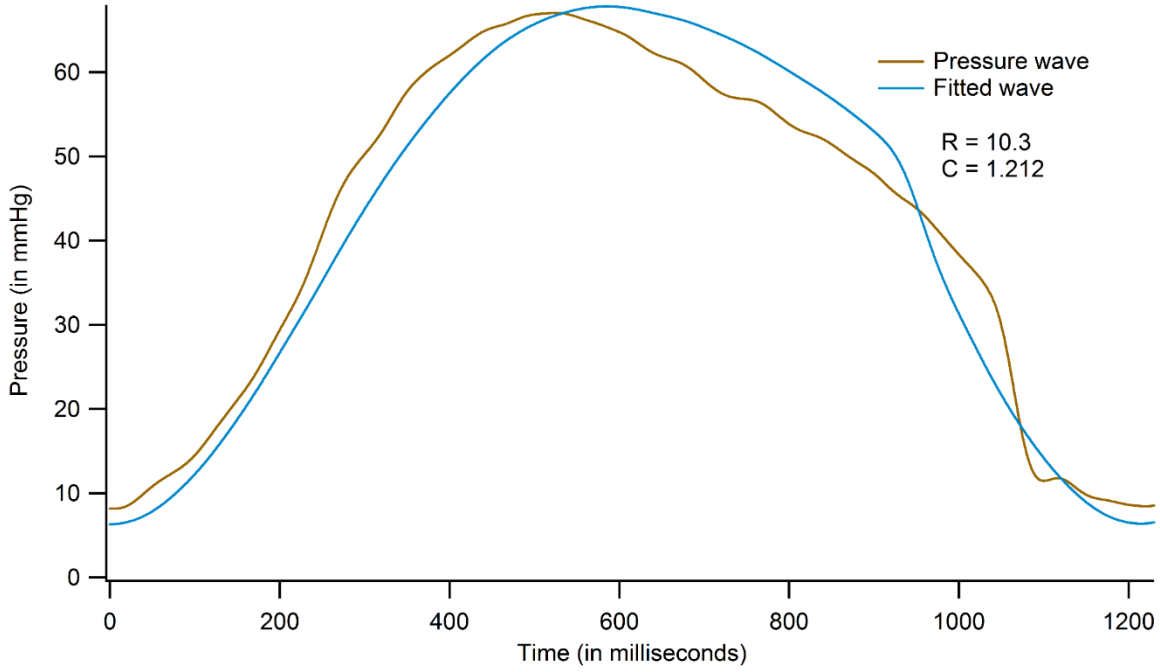


Figure 11: A pressure wave from a normal recording and a fitted wave is shown .The derived resistance was 10.3 mmHg.ml^{-1}.min and compliance was 1.212 ml.mmHg^{-1}.

Results & Discussion

Resistance and capacitance wave forms derived from pressure and flow wave forms changed predictably upon addition of KCl.

Increased KCl in the extracellular space causes depolarisation of cells and opening of voltage gated Ca^{2+} channels. So administration of a high concentration KCl solution will result in contraction of smooth muscle cells. This happens both in the large arteries and the arterioles. So the expected result was an increase in peripheral resistance (due to contraction of arterioles) and a decrease in compliance (due to contraction of smooth muscle of large arteries).

Pressure recordings obtained in one of the experiments with administration of KCl is shown below

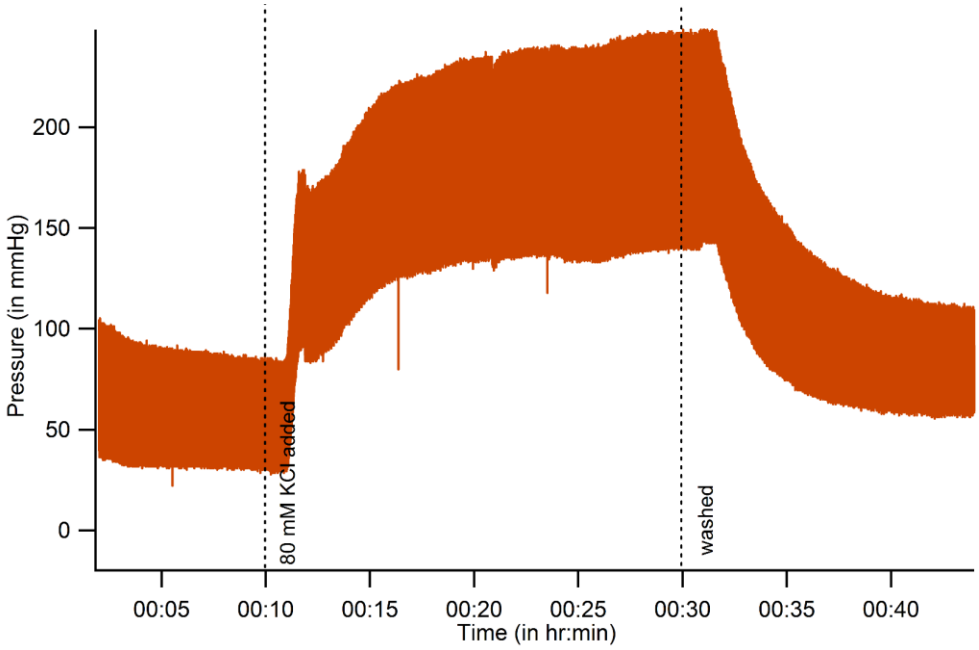
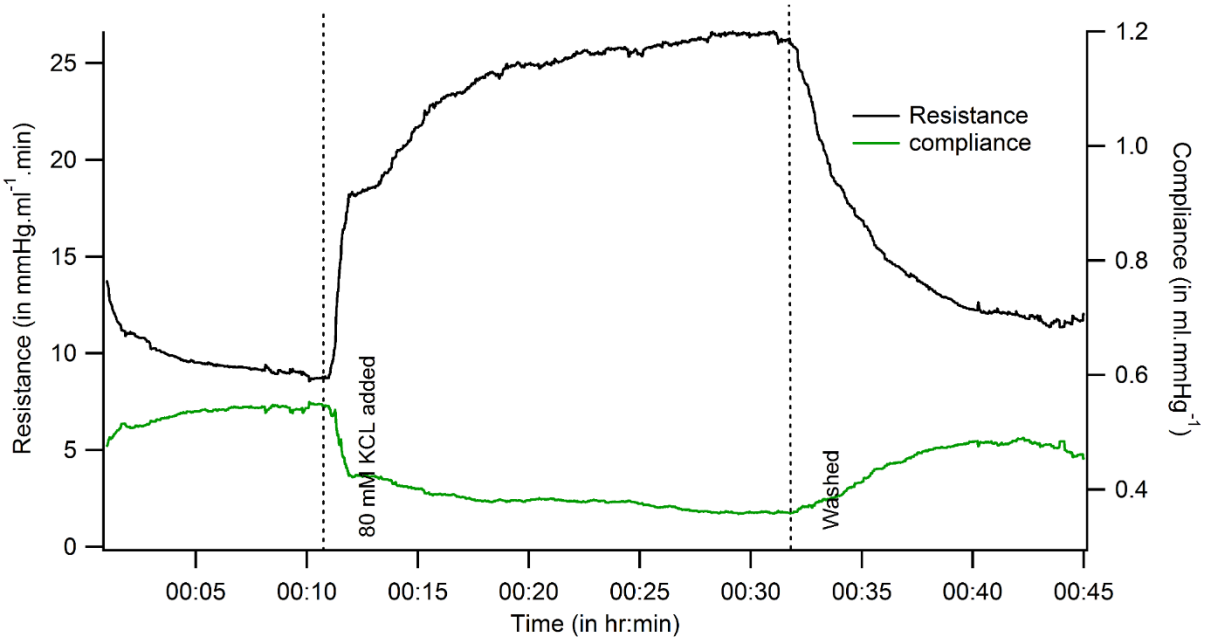


Figure 12: Pressure recordings from one experiment where KCl was administered. The normal physiological salt solution was switched with a high KCl (80 mM) solution at 10 minutes and the recording continued for 20 minutes. The solution was then switched back to normal

The pressure recording showed an expected rise in blood pressure on administration of KCl.

The parameters Resistance (R) and Compliance (C) derived from the above pressure recording is shown below:



80 mM KCl caused increase in resistance and decrease in compliance. The effect immediately reversed on washing with normal solution. This was an expected effect from high concentrations of KCl.

Resistance values obtained from 3 experiments averaged over 5 min duration

before intervention	After intervention		After wash	
Mean resistance	Mean resistance	Percentage increase from initial values	Mean resistance	Percentage increase from initial value
8.9293	25.6182	186.9	11.9881	34.26
11.2383	35.4824	215.73	11.0577	-1.60
11.6501	25.5309	119.15	9.1940	-21.08

Compliance values obtained from 3 experiments averaged over 5 minutes

before intervention	After intervention		After wash	
Mean compliance	Mean compliance	Percentage increase from initial values	Mean compliance	Percentage increase from initial values
0.543515	0.379119	-30.25	0.482668	-11.2
1.61589	0.878452	-45.64	1.32249	-18.16
1.01266	0.676209	-33.22	0.939515	-7.2

Effect of Adrenalin

High doses of adrenaline cause vascular smooth muscle contraction by its effect on α1 receptors. So the expected result was an increase in resistance and decrease in compliance.

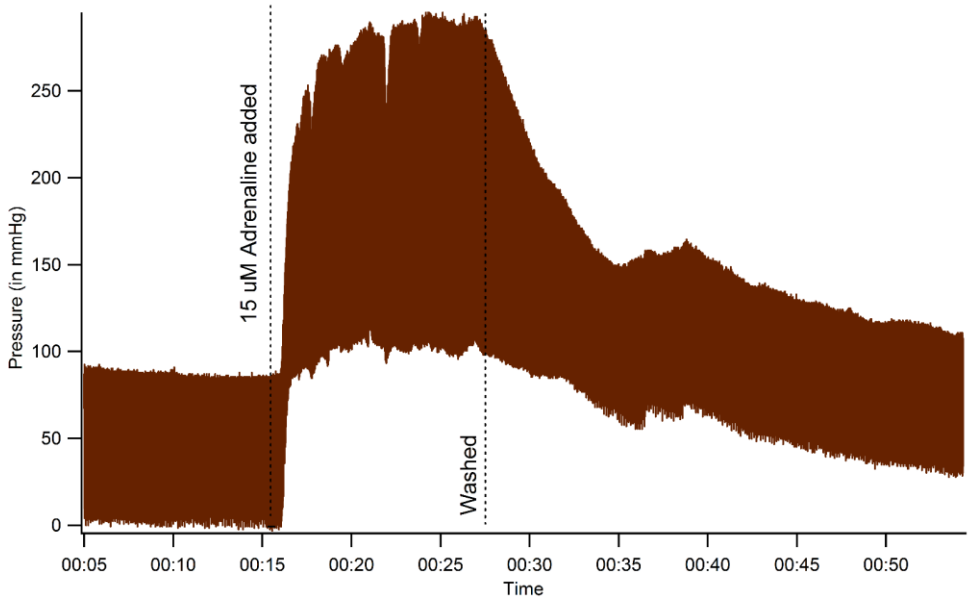
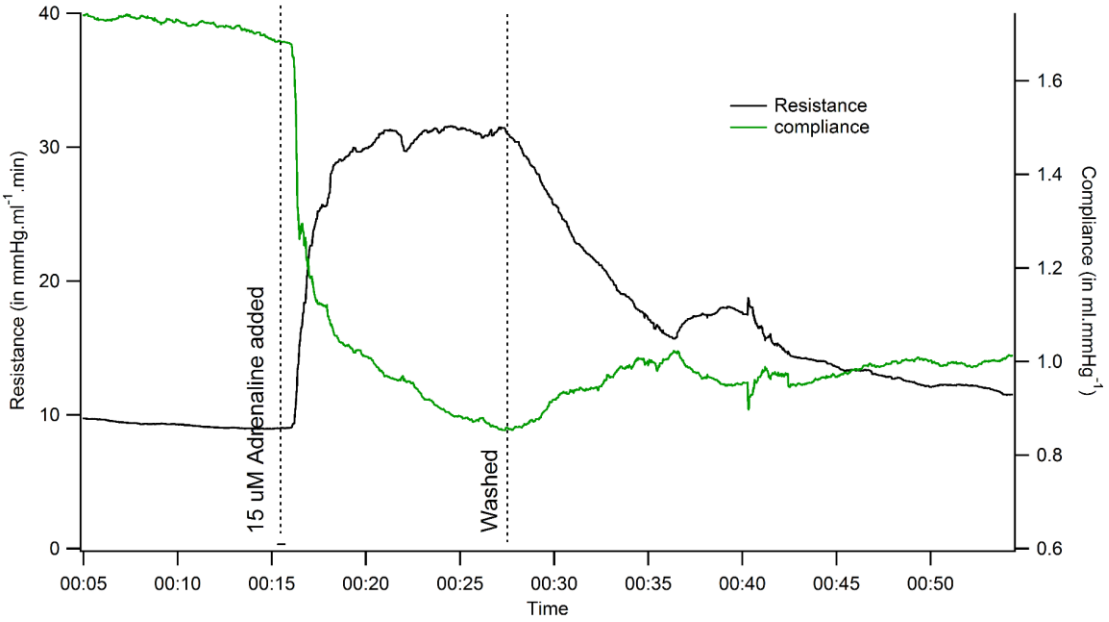


Figure 13: Blood pressure recording in an experiment with Adrenalin. 15μM adrenalin was added at 15 minutes and washed at 27 minutes.

Blood pressure predictably increased on administration of adrenalin.

The resistance and compliance derived from the above pressure curve is shown below:



Resistance values obtained from 3 experiments averaged over 5 min duration

before intervention	After intervention		After wash	
Mean resistance	Mean resistance	Percentage increase from initial values	Mean resistance	Percentage increase from initial values
8.68337	28.1507	224.19	20.4731	141.41
9.10167	29.4835	223.94	16.5961	82.3
9.89092	37.2167	276.27	29.1683	195.90

Compliance values obtained from 3 experiments averaged over 5 minutes

before intervention	After intervention		After wash	
Mean compliance	Mean compliance	Percentage increase from initial values	Mean compliance	Percentage increase from initial values
0.629727	0.372106	-40.91	0.379684	-39.7
1.72061	0.865909	-49.67	0.997477	-42.03
0.768342	0.424927	-44.71	0.438444	-42.94

15 μM concentration of adrenalin was perfused and it produced a similar change to that of KCl. There was an increase in resistance and decrease in compliance. However, the duration of effect was longer.

While the values of resistance dropped by 60% after 5 minutes in KCl experiments, the resistance drop was 30% after wash of adrenalin. The resistance slowly decreased and took about 30 minutes for the values to return to normal. The compliance changes were still slow and persisted at low levels for longer durations. The compliance values increased by only 8% after wash, while resistance recovered faster.

This observation points to a differential effect of adrenaline in large and small arteries. Rajkumar et al.[25] had shown that in rabbit, phenylephrine had a higher affinity to longitudinal muscles of aorta, than the circular muscles of fallopian tube.

This distinction in affinity may explain our findings where the affinity to longitudinal muscles of aorta is higher and leads to prolonged reduction in compliance.

Compliance is an important parameter

Compliance is usually regarded as a constant value and there is a paucity of studies regarding compliance as an important determinant of momentary changes in flow and pressure. In our study, we observed momentary changes in compliance which occurred in shorter durations.

It becomes necessary to study compliance as an important parameter in determining changes in blood pressure.

Validation of resistance

Resistance was derived from pressure recording using methods mentioned in the previous section. Resistance was also calculated using the relationship Mean Blood Pressure / Flow. A comparison between the resistance derived and the calculated resistance in one of the experiments is shown below

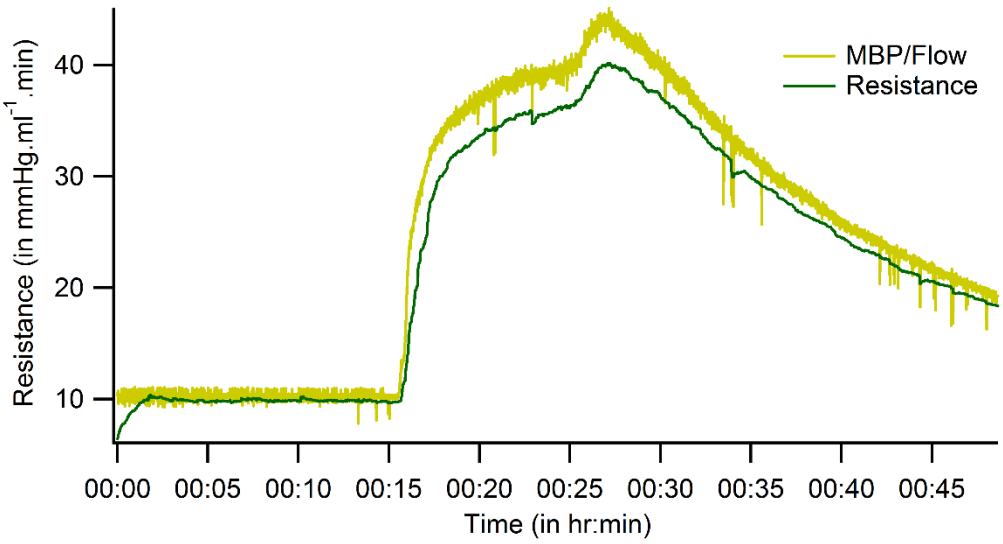


Figure 14: comparison of resistance derived from windkessel equation and resistance derived from Mean Blood Pressure/Flow in an experiment where adrenaline was administered.

Pearson's Correlation coefficient was calculated between the derived resistance and resistance calculated from the mean blood pressure and flow. The values obtained from six experiments were

	Pearson's Correlation coefficient
Adrenalin	0.974
	0.987
	0.995
KCl	0.99
	0.878
	0.99

The mean ± SD of the Pearson's correlation coefficient was 0.97 ± 0.04.

Resistance derived using the windkessel equation correlated well with resistance derived using the equation Mean Blood Pressure / Flow. Thus the derived resistance can accurately predict changes in the system.

The curve below depicts the correlation between the derived resistance and the resistance obtained from the mean blood pressure in one of the recordings

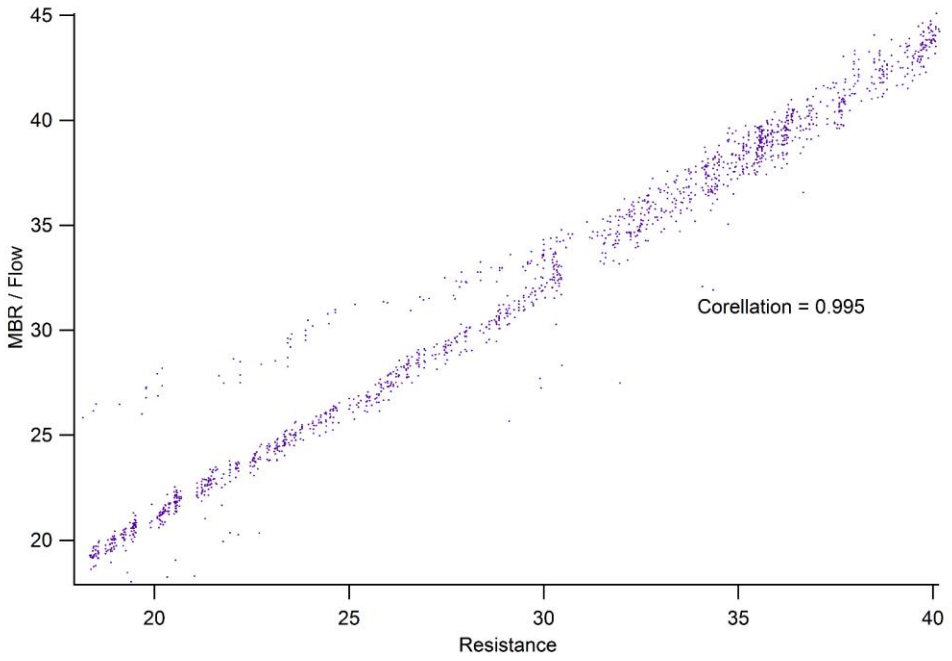


Figure 15: Graph with derived resistance along the X-axis and resistance calculated from mean blood pressure along the Y-axis. The values were obtained from an experiment where adrenalin was used. The plot shows a straight line with a slope closer to 1 depicting a perfect correlation of the values. The Pearson/s correlation value was found as 0.995.

Mean percentage error was calculated between the two different resistances. The values obtained were shown below.

	Mean Absolute Percentage Error
Adrenalin	5.52 %
	12.41 %
	7.22 %
KCl	10.4 %
	13.1 %
	5.01 %

The mean ± SD of the Mean Absolute Percentage error was 8.9 ± 3.5%.

There was an 8.9% mean absolute difference in the values derived. The derived values were always lower than the values from Mean Blood Pressure / Flow. This may be because the two element windkessel model does not include additional parameters like impedance and inductance which are part of three element and four element models.

Rat hind limb Preparation

The rat hind limb preparation was chosen because it includes a subset of the arterial system with the large arteries, small arteries and arterioles, thus including both resistance and capacitance vessels. The model could show the differential response in resistance and capacitance vessels and could be useful in studies of effect of drugs on the vascular system, in the absence of neural influences.

In experiments with KCl and Adrenalin, the preparation showed the expected results which was contraction of the vessels and increase in resistance.

Limitations

A two element windkessel model was used in place of a three or four element windkessel model for computational ease. The resistance derived in this study underestimated the actual resistance by approximately 9%. This difference may have been avoided if higher degree models had been used. But it is also argued that higher models may have their own disadvantages. Three element windkessel model may underestimate compliance by 25% in comparison to two element model[23].

Summary

Two element windkessel model was used to derive resistance and compliance from recorded pressure.

Continuous arterial pressure was recorded from a rat hind limb preparation. A flow curve was estimated using pressure curve for use with the windkessel model.

An algorithm was developed to derive resistance and compliance from the two recordings mentioned above, namely arterial pressure and flow. The algorithm assumed a simple vascular system with the two element windkessel model involving a resistance and capacitance. The flow wave was curve fitted to the pressure wave and the parameters, Resistance and compliance were derived which gave the best fit.

Resistance which was derived correlated well with the resistance measured from mean blood pressure and flow but the resistance was underestimated by around 9%.

Compliance derived from the model was not a constant value and varied with time.

Adrenalin which was used to elicit changes in resistance and compliance showed differential responses during recovery after drug was removed. This is hypothesised to be because of differential effects of adrenaline on longitudinal and circular smooth muscles.

Conclusion

Rat hindlimb preparation is a reliable preparation to study dynamics of vascular system and to study effects of drugs on the vascular system in absence of regulatory mechanisms.

Windkessel model of the vascular system is a reliable model to study peripheral resistance and arterial compliance. A simple two-element windkessel model can predict changes in peripheral resistance with high degree of accuracy.

Compliance of arteries is not a constant value and momentary changes can occur in short durations. So, Compliance is an important determinant of changes in flow and pressure.

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Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

September 21, 2015

Dr. Benjamin Jebaraj
PG Registrar
Department of Physiology
Christian Medical College
Vellore 632 002

Sub: **Fluid Research Grant Project:**
Validation of an algorithm to estimate vascular resistance and compliance in a perfused rat hind limb preparation.
Dr. Benjamin Jebaraj (Employment Number: 20962), PG registrar, Physiology, Dr. Sathya Subramani, Employment Number: 14123, physiology.

Ref: IRB Min No: 9521 [01HERS] dated 07.07.2015

Dear Dr. Benjamin Jebaraj,

I enclose the following documents:- 1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. NIHAL THOMAS
MD,MNAMS,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
Vice - Principal (Research) - Reg. No. 43983
Christian Medical College, Vellore - 632 004.

Dr. Nihal Thomas
Secretary (Ethics Committee),
Institutional Review Board

Cc: Dr. Sathya Subramani, Department of Physiology, CMC,

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
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September 21, 2015

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PG Registrar
Department of Physiology
Christian Medical College
Vellore 632 002

Sub: **Fluid Research Grant Project:**
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Dr. Benjamin Jebaraj (Employment Number: 20962), PG registrar, Physiology,
Dr. Sathya Subramani, Employment Number: 14123, physiology.

Ref: IRB Min No: 9521 [OTHERS] dated 07.07.2015

Dear Dr. Benjamin Jebaraj,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Validation of an algorithm to estimate vascular resistance and compliance in a perfused rat hind limb preparation" on July 07th 2015.

The Committee reviewed the following documents:

1. IRB Application format
2. Cvs of Drs. Benjamin Jebaraj and Sathya Subramani
3. No of documents 1 – 2

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on July 07th 2015 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Alfred Job Daniel	D Ortho, MS Ortho, DNB Ortho	Principal, Chairperson-Research Committee, IRB, CMC, Vellore	Internal, Clinician
Dr. Nihal Thomas	MD, MNAMS, DNB(Endo), FRACP (Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology, Additional Vice Principal (Research), Deputy Chairperson(Research Chairperson), Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician
Dr. Simon Pavamani	MBBS, MD	Professor, Radiotherapy, CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS, MCh	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician
Dr. Bobby John	MBBS, MD, DM, PhD, MAMS	Professor, Cardiology, CMC, Vellore	Internal, Clinician
Dr. Benjamin Perakath	MBBS, MS, FRCS	Professor, Colorectal Surgery, CMC, Vellore	Internal, Clinician
Dr. Chandrasingh	MS, MCH, DMB	Professor, Urology, CMC, Vellore	Internal, Clinician
Dr. Anup Ramachandran	PhD	The Wellcome Trust Research Laboratory Gastrointestinal Sciences, CMC, Vellore	Internal, Basic Medical Scientist
Dr. Anand Zachariah	MBBS, PhD	Professor, Medicine, CMC, Vellore	Internal, Clinician

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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. Visalakshi, J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Rajesh Kannangai	MD, PhD.	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Dr. Niranjan Thomas	DCH, MD, DNB (Paediatrics)	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Dr. Jacob John	MBBS, MD	Associate Professor, Community health, CMC, Vellore	Internal, Clinician
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Validation of an algorithm to estimate vascular resistance and compliance in a perfused rat hind limb preperation." on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in)

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Additional Vice Principal (Research)

Fluid Grant Allocation:

A sum of Rs. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 1 year and out of which a maximum of Rs 5,000/- can be spent for stationery, printing, Xeroxing and computer charges (if computers used are within the institution).

Yours sincerely,

Dr. NIHAL THOMAS
MD, MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin), FRCP(Glasg)
Vice - Principal (Research) - Reg. No. 43003
Christian Medical College, Vellore - 632 002.

CHRISTIAN MEDICAL COLLEGE VELLORE INDIA

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Sathya Subramani, Professor, Department of Physiology, CMC, Vellore

IRB Min No: 9521 [OTHERS] dated 07.07.2015

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