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A Study Of Hemolysis In Pulsatile Blood Pumps

By

Mohamad A.M. Alami

A thesis submitted for the degree of Doctor of Philosophy of The University of Edinburgh.

To my dear parents,
my beloved wife,
and my family, particularly the boundless
love and courage of my sister Lamis and
her late husband, Anan.
Acknowledgements

I am very much indebted to my supervisor, Dr. Norman Macleod, for his guidance and encouragement throughout the course of this work. His kindness and the friendship of his family are very much appreciated by my wife and myself.

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I acknowledge, with gratitude, the skill and craftsmanship of Mr. R. Hardy who constructed the actuator, the valves and the pump chambers used in this work. His help has been invaluable.

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Abstract

The aim of this study is to investigate the main sources of hemolysis in a pulsatile blood pump by hemolytically comparing various designs and materials of construction of the pump chamber and its valves.

The pump employed is of the positive displacement type, driven by an actuator whose parameters, viz. frequency, stroke volume and systolic/diastolic time ratio, can be independently varied. The dynamic characteristics of the resulting flow output (i.e. flow spectra) are very similar to those of the corresponding output of the human left ventricle.

In the design of a mock arterial tree, the reproduction of the physiological systemic input impedance necessarily involves large surface areas exposed to blood, a high surface/volume ratio and relatively high levels of wall shear stress - factors believed to contribute appreciably to RBC trauma. A mock arterial tree of favourable hemolytic characteristics but of relatively higher input impedance, has been constructed and successfully used in the hemolytic experiments.

Using fresh greyhound and sheep blood, experiments have been carried out to develop reliable procedure for the intended hemolytic studies. Of these, the time dependence of the fragility of the RBC membrane proved to be the most significant.

The design differences in the various studied blood chambers have resulted in the attainment of (a) various levels of fluid shear stress developed at the surfaces of the chamber and (b) various degrees of blood stasis within the chamber. These differences have been found to be hemolytically insignificant. Design and material changes in the occluders of the various compared valves have been found to result in appreciable hemolytic differences. The results suggest that for a given material of construction, the trauma produced by the valve is very much affected by the design of its occluder.
Furthermore, differences in the traumatic properties of various materials seem to depend on the 'severity' of the flow conditions. Of the two compared materials, pyrolytic carbon has been found to be appreciably less hemolytic than delrin. The hemolytic properties of delrin have been found to be unaffected by changes in its surface roughness.
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CHAPTER ONE

INTRODUCTION

The design of pulsatile blood pumps and prosthetic heart valves is mainly directed at overcoming the important problems of thrombosis and hemolysis resulting from the exposure of flowing blood to such devices. Although both types of blood damage can result from the use of extracorporeal circuits and can both occur in certain diseases (e.g. microangiopathic hemolytic anemia), there is as yet little evidence of a direct relationship between them (1). Red Blood Cell (RBC) damage, sublethal and outright rupture, associated with the above mentioned devices is generally considered to be less important than thrombosis. RBC trauma, however, cannot be ignored when designing blood pumps for permanent implantation or for use as long term assist devices.

The aim of this study is to investigate the main sources of hemolysis in a positive displacement pulsatile pump. The literature, reviewed below, suggests that damage to the RBC may be broadly attributed to the effects of fluid forces, resulting from the flow of blood, acting directly on the cell membrane (a) remote from the non-biological surfaces and (b) in the vicinity of these surfaces. For a given flow rate, the developed fluid forces depend on the design of the pump chamber and its inlet and outlet valves. The work presented here has therefore consisted of hemolytic comparisons between various designs and materials of
construction of the pulsatile pump and its valves.

Three blood chambers whose design differences were thought to be hemolytically significant have been constructed. These design differences result in the attainment of (a) various levels of fluid shear stress developed at the surfaces of the chamber and (b) various degrees of blood stasis within the chamber. The type of valve employed, the Edinburgh Valve (57), has been specifically designed for use in pulsatile blood pumps. As with the prosthetic form of this valve (currently being evaluated in animal experiments), the design of the occluder of the valve may be varied. Design and material differences in the valves of the blood chamber have been effected by varying the designs and the materials of construction of their occluders. The adopted designs result in the attainment of various degrees of blood turbulence in the vicinity of the occluders and at their surfaces. The materials that have been used in constructing the valve occluders are Delrin (a form of acetal resin) and Pyrolytic Carbon. Both have been separately used in the construction of various types of clinically employed prosthetic valves.

The pump, its inlet and outlet valves and the driving mechanism have all been designed and constructed in this department. The pulsatile pump consists of two chambers, the blood and the hydraulic chambers, separated by a flexible diaphragm. The motion of the diaphragm is predetermined by the controlled flow of an incompressible fluid into the hydraulic chamber. The flow of this fluid is controlled by the driving actuator (57,58) whose parameters, viz. frequency, stroke volume and systolic/diastolic
time ratio can be independently varied.

When driven with the above mentioned actuator, the pulsatile pump produces a flow output whose dynamic characteristics are very similar to those of the physiological output. The pump may be loaded with a mock arterial tree whose impedance is equivalent to the physiological systemic impedance. The resultant mock cardiovascular loop may then be used as a test rig for examining the performance of a prosthetic valve and the factors that influence its behaviour, e.g. design of the occluder and the geometry of the flow passages in the immediate vicinity of the valve. However, as discussed in Chapter Three, the high degree of blood trauma expected from such a loop renders it unsuitable for use in hemolytic studies. A mock arterial tree of low traumatic characteristics has therefore been designed and successfully used in the conducted hemolytic experiments.

Both fresh blood obtained from various slaughtered animals and outdated human blood were evaluated for use in the intended hemolytic experiments. As discussed later, these types of blood were found to be unsuitable. Unfortunately and with sincere apologies to animal lovers, it proved necessary to use fresh blood by exsanguinating greyhounds and sheep in order to obtain consistent results. Using this blood, a series of experiments was carried out to develop a reliable procedure for the intended hemolytic studies. These included experiments on heparinisation, haemodilution, siliconisation of surfaces, the effect of blood-air contact and the time dependence of the fragility of the RBC membrane. The last of these factors, referred to as the RBC ageing effect, proved to be the most significant.
CHAPTER TWO

A LITERATURE REVIEW OF THE MECHANICAL SOURCES OF
DAMAGE TO THE RED BLOOD CELL

2.1 Introduction

The use of extracorporeal circuits and prosthetic devices may inflict various degrees of damage on the RBC membrane; (a) in the extreme case, these devices cause the cell to rupture, thus releasing intracellular hemoglobin into the surrounding plasma; (b) the cell, though unruptured, undergoes various alterations in its 'biochemical' contents. Nevertheless, these sub-hemolytically damaged cells are prematurely removed from the body.

Hemolytic damage to the cell (i.e. cell rupture) results in an increase in the free plasma hemoglobin concentration of the blood. Although the body organs, e.g. spleen and liver, can dispose of small quantities of the free hemoglobin, a certain level is reached beyond which the free hemoglobin starts to accumulate and become toxic to the body. Also, when the rate of cell generation is exceeded by the rate of cell destruction, there results a decrease in the total number of healthy cells, which may cause patients to become anemic.

Sub-hemolytic damage to the RBCs has been studies by one, or a combination of the following:

(1) In-vivo, life span studies on sub-hemolytically damaged cells: a significant reduction in the half-life of the damaged cells, tagged with Cr$^{51}$ isotope, has been reported by various workers (2-5)

(2) A visual comparison between the sizes and shapes of healthy and sub-hemolytically damaged cells, via a scanning electron micro-
scope: the damaged cells have been observed to have distorted shapes, reduced or swollen sizes (6,7).

(3) A study of the biochemical changes undergone by the damaged, yet unruptured cells: e.g. (a) delayed release by the cell of Lactic dehydrogenase isozymes (8) and (b) chemicals considered vital to membrane stability, e.g. ATP and 2,3-DPG, have been reported to leak out of the damaged cell (9,4,10).

In the flow of blood in extracorporeal circuits and prosthetic devices, mechanical damage to the RBC may be broadly attributed to the effects of the fluid forces, adequately described by the fluid shear stress, acting directly on the cell membrane (a) remote from non-biological surfaces and (b) in the vicinity of these surfaces.

2.2 Direct Shear Stress Effect on RBC Membrane

Essentially, the literature reports two main methods for investigating the magnitude of the shear stress that a RBC can sustain without rupturing. The first method utilises the concentric cylinder viscometer (2,13,14,15,16), and the second, subjects blood to the jet test (4,12,17). The differences in the results from the two methods have, to some extent, now been satisfactorily resolved.

2.2.1 Viscometric Measurements

The most thorough work with the rotational viscometer has been done by Hellums and co-workers (2,13). In their early work, (2), they subjected blood to a known shearing stress of $\sim 3000 \text{ dynes/cm}^2$, when applied for a duration of 2 minutes. Hellums et al have also shown that when the viscosity of the cell
suspensing medium is increased two fold, the results remain unchanged. From this, they conclude that, under the prevailing conditions in their viscometer, the degree of damage sustained by the cells is a function of the magnitude of the applied shearing stress and is unaffected by the shearing rate.

In-vivo studies by the same investigators on cells tagged with Cr$^{51}$ isotope, have shown that unruptured cells do suffer reduced life spans, after having been sheared by a stress of 1500 dynes/cm$^2$ for the duration of 2 minutes. The cell life span has also been found to drop sharply as cells are sheared at higher stress levels.

In their more recent and thorough work, Hellums et al (13) have investigated several factors that might complicate the interpretation of their viscometric results. These factors include solid-surface interaction, centrifugal forces acting on cells, air-interface interaction above the blood-filled gap, mixing between sheared and unsheared layers, cell-cell interaction and the effect of viscous heating. The results do indicate that the various factors, listed above, have negligible effects, and show that there is a threshold shear stress level, $\sim 1500$ dynes/cm$^2$ (applied for 2 minutes), above which the rate of cell rupture becomes significant. The rate of hemolysis in their viscometric experiments, they conclude, is directly due to the applied shear stress, and would take place whether cells are sheared remote from, or in the vicinity of non-biological surfaces.

The most disputed of the above listed factors seems to be the cell-surface interaction. Blackshear (18) maintains that at such high levels of shear stress, cell diffusion towards the
walls may be augmented, and that most of the observed cell damage may be attributed to cell-surface interaction. It is maintained that in the vicinity of surfaces, far lower magnitudes of shear stress ($<1500 \text{ dynes/cm}^2$) would be sufficient to hemolyse surface-colliding cells. Such criticism does not seem to be justified in the case of Hellum's experiments, principally for the following reasons:

(1) Hellums found that the threshold hemolytic value of 1500 dynes/cm$^2$ and the pattern of hemolysis with increasing levels of shear stress remain the same when the surface/volume ratio of their viscometer is changed by a factor of 2.7. Now, if surface effects were to play a significant hemolytic role in the above experiments, the degree of hemolysis would be expected to increase upon increasing the surface/volume ratio. This however is not borne out.

(2) Blackshear (18) reports that cell-wall interactions increase markedly as the hematocrit exceeds 40%. The results of experiments discussed in Chapter (4), and conducted by the author (M.A.A.) suggest similar findings. If surface interactions in Hellums's viscometer are significantly hemolytic, then they would be particularly evident at high hematocrit levels ($\sim40\%$). In experiments with the rotating viscometer, Hellums (13) have used blood specimens with hematocrits varying between 0.3% and 60%. The specimens have been subjected to a constant shear stress of 3000 dynes/cm$^2$ for equal time durations (2 minutes). The change in the percentage hemolysis (i.e. the percentage of the free plasma hemoglobin as a fraction of the total hemoglobin in the whole blood), in the various sheared specimens has been
found to be insignificant.

It is worth noting that aggregations of cells in blood which is sheared at high stress levels are very unlikely to occur. In their studies on the forces between RBCs when sheared in a cone-plate viscometer, Schmid-Schonbein et al (19) have observed that rouleaux formations, initially present in the unsheared blood, do disperse when the applied fluid stress exceeds 4 dynes/cm$^2$.

The findings of Sutera et al (14) closely agree with those of Hellums and co-workers. In their experiments, Sutera et al (14) have used an optical method for the continuous monitoring of hemolysis in their concentric cylinder viscometer. The optical system is based on the light-scattering ability of intact erythrocytes and records the increasing transparency of the suspension as cells hemolyse. (When measurements taken with the optical system are compared with a standard method of hemoglobin determination, presumably the benzidine method, there results a discrepancy of the order of 10% of the measured values.) Although this new method requires further investigation, the results nevertheless do indicate that the onset of significant levels of hemolysis ($\sim 1\%$ hemolysis) takes place after sufficiently long exposure ($\sim 10$ minutes) of the blood samples to a shear stress of 1500 dynes/cm$^2$. Above this level, the percent hemolysis is found to increase sharply with rising stress values, e.g.:

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Sutera's results (14) do also show that for shear stress levels above 1500 dynes/cm² the percent hemolysis rises proportionately with increasing time durations of the applied stress. For lower stress levels, ~10² dynes/cm², longer time durations may be needed to cause any significant levels of hemolysis, e.g. when blood is sheared at 500 dynes/cm² for a period of two hours, the resulting degree of cell rupture is <0.5% hemolysis. However, using an electron microscope, Sutera et al (14) have observed that at the end of this period, a significant fraction, ~1/7, of the unruptured RBCs have distorted shapes, probably indicating a form of sub-lethal damage.

In their rotational viscometer, Shapiro et al (15) sheared blood at a stress level of 500 dynes/cm², applied for a period of 3 minutes. They found that the ruptured cells did not exceed 0.2% of all sheared cells. Shapiro and co-workers suggest that a significant part of this hemolysis is due to surface related effects in their viscometer. As the cells used in their work were obtained from outdated human blood, it seems reasonable to assume that much lower hemolysis levels would be obtained if fresh blood had been used instead, (see Chapter 4, on outdated human blood).

2.2.2 The Jet Test

The jet test involves the injection, at high velocity, of a fixed volume of isotonic saline, plasma, or blood into an initially stagnant reservoir of blood or cell suspensions. RBCs entrained near the conical boundary of the jet are subjected to high levels of shear stress. Of major advantage in this test,
is the fact that cells are subjected to the stress when remote from the non-biological surfaces.

For the exact relationship between the shear stress and the centre line velocity, $U$, i.e. the maximum velocity in the jet, a knowledge of the relevant value of the eddy diffusivity, $\varepsilon$, is required. From Schlichting (20), the turbulent shear stress, $\tau$, in a jet is given by:

$$\tau = \rho \varepsilon \frac{du}{dr}$$

where $\rho$ = density of cell suspension, $\frac{du}{dr}$ = shear rate.

For the jet, $\varepsilon$ can be evaluated (using Prandtl's mixing length theory) by:

$$\varepsilon = \chi b \left( u_{\text{max}} - u_{\text{min}} \right)$$

where $(u_{\text{max}} - u_{\text{min}})$ is the maximum difference in the time-mean flow velocity in the width of the mixing zone, $b$. $\chi$ is an experimentally determined dimensionless number. Bernstein et al (4) maintain that the maximum stress level developed in the jet is proportional to the square of the jet centerline velocity, $U \text{ (cm/sec.)}$, viz.:

$$\tau_{\text{max}} = 0.03 \rho U^2 \text{ dynes/cm}^2.$$

The reported experiments (4,12,17) however, do not clarify how this relationship is derived. But nevertheless, it is used to obtain the maximum level of stress produced by the jet.

The values of threshold shear stress (i.e. stress at which a significant degree of hemolysis occurs) derived from the reported jet tests are of the order of $10^4$ dynes/cm$^2$. The estimated duration of the applied stress is $10^{-5}$ seconds (4,12,17). Forstrom (21) is reported to have obtained the same results when either saline, plasma or blood are injected into stagnant blood. This, it is suggested, indicates that the hemolysis produced is independent of the shear rate - a conclusion reached by Hellums (2).

The reliability of the jet test cannot be satis-
factorily accepted without resolving the problem of experimentally deriving an accurate relationship between the jet's centre line velocity and the maximum developed shear stress. As the reviewed works do not show how this relationship is obtained, the reliability of the reported results can only be tentative. What may be inferred from the jet test is that the RBC may hemolyse when subjected to very high levels of shear stress when applied for very brief time durations, even at locations far from non-biological surfaces.

2.2.3 Other Tests

Rooney (22) has observed that when RBCs are subjected to the shear field generated by an ultrasonically pulsating gas bubble, the onset of hemolysis occurs at an estimated stress value of $\sim 4500$ dynes/cm$^2$, applied for the short duration of $\sim 10^{-2}$ seconds. Williams et al. (23) have found a threshold of $\sim 5600$ dynes/cm$^2$, applied for $\sim 10^{-3}$ seconds, when RBCs are subjected to a shear field in the neighbourhood of an oscillating wire.

The discrepancies in the results of the various methods used for establishing the threshold shear stress values, may be resolved by recalling the viscoelastic nature of the RBC membrane (11). This implies that the exposure times of the applied stress levels need to be considered. The following table enlists a summary of the above reviewed methods:
As Helium suggests (13), different workers have apparently studied different regimes of the exposure time-stress domain. Due to the viscoelastic property of the RBC membrane, the threshold stress value thus increases with decreasing time duration of the applied stress. The viscoelastic nature of the membrane is clearly shown by the findings of Rand (11), as described below.

### 2.3 Effect of Applied Tensile Stress on The RBC Membrane

The effect of subjecting the RBC membrane to a tensile stress has been investigated by Rand (11). The technique employed required the measurement of the pressure and the time needed to lyse a cell by sucking it into a micropipette. When a long tongue of the cell's membrane is pulled into the pipette, the membrane of the RBC spontaneously collapses on itself, thereby producing a small globule and a swollen red cell. If, initially, the cells are swollen enough, by immersion in a hypotonic solution, they cannot afford enough surface area for a long tongue to move into the micropipette. When these cells are stressed for a short period of time they hemolyse. Rand's results suggest that the magnitude of the tensile stress, required for cell lysis, drops sharply with increasing time durations of the applied stress. At

<table>
<thead>
<tr>
<th>Threshold Shear Stress</th>
<th>Duration of Stress</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 x 10^3 dynes/sq.cm.</td>
<td>1.2 x 10^2 seconds</td>
<td>Rotational viscometer (2,13,14)</td>
</tr>
<tr>
<td>4.5 x 10^3 dynes/sq.cm.</td>
<td>10^-2 seconds</td>
<td>Pulsating gas bubble (22)</td>
</tr>
<tr>
<td>5.6 x 10^3 dynes/sq.cm.</td>
<td>10^-3 seconds</td>
<td>Oscillating wire (23)</td>
</tr>
<tr>
<td>5.5 x 10^4 dynes/sq.cm.</td>
<td>10^-5 seconds</td>
<td>Jet test (4,12,17)</td>
</tr>
</tbody>
</table>
relatively low levels of tensile stress, < 12 dyne/cm, longer
time durations, 25 secs. - 2.5 mins., are needed to lyse the cells.

Rand (11) maintains that the pattern of hemolysis
is consistent with a model of the membrane behaving as a visco-
elastic solid in which hemolysis occurs, after a certain time
duration, when the stress exceeds some limiting value, $T_c$.
Furthermore, this critical value is a decreasing function of the
duration of the applied tensile stress. This viscoelastic property
of the membrane, as investigated by Rand, seems to be consistent
with the findings, discussed above, describing the direct effect
of fluid shear stress on the RBC.

2.4 Shear Stress and RBC - Non-Biological Surface Interactions

The outcome of cell-surface interaction, even for
the simple flow of blood in a tube may be modified by two main
factors: the nature of the blood-exposed surface and the flow
conditions within the tube. The former may include the type
and roughness of the surface, its electrochemistry, type of
coating and nature of any deposits that may 'contaminate' the
surface during the blood flow. The latter will dictate the
distribution of cells within the tube, particularly in the
vicinity of the surface, the rate of cell-surface collisions,
the 'severity' of these collisions, and the duration of cell-
surface contact. The hematocrit of the flowing blood will also
affect the rate of cell-surface collisions.

The few and scattered references, reviewed
below, do not offer a systematic treatment of the outcomes and
mechanisms of cell-wall interactions. In an attempt to utilise
the little information that is available, a general approach to
the analysis of the likely mechanisms of these interactions is
proposed.

In the vicinity of a non-biological surface, of
whatever configuration, there exists a region of the fluid flow,
within which a RBC is most likely to collide with the surface.
This is illustrated in Figure (2.1) for a cell moving with a
velocity $u$, close to the various probable types of surfaces. The
cell-wall collision rate will depend on the number of cells within
this region and their velocities with respect to the surface. For
a RBC with velocity $u_y$ normal to the surface, and $u_x$ parallel to
the surface, the probable outcomes of the collision processes are:

(a) For low values of $u_y$, the colliding RBC may approach the
surface slowly enough to allow adhesive forces to bind it
to the wall. The continued cell-wall attachment may cause the
RBC to undergo some form of sub-lethal damage, which is likely
to depend on the nature of the surface involved. The adherent
cell may also be subjected to a wall shear stress which can be
either constant, as in steady laminar flow, or variable
in magnitude and direction, as in pulsating and turbulent
steady flows. The detachment of the adherent RBC may
probably result in the rupture of the membrane or contribute
further to any sub-lethal damage sustained by the detached cell.
Alternatively, probably for the case of a smooth wall, the
fluid shear stress may cause the adherent cell to be 'dragged'
along the surface.

(b) The colliding cell, on the other hand, may 'bounce off' and
be reflected away from the surface. The RBC may sustain some
smooth surface
rough surface
surface covered with deposits, particles, etc., e.g. platelets
surface coated with plasma etc.

Figure (2.1): A RBC flowing in the vicinity of some of the likely surfaces with which it collides in extracorporeal circuits.
damage during its brief encounter with the wall.

(c) The colliding cell, particularly at very high values of $u_y$, may rupture on impact with the surface.

The hemolytic effects of surfaces, according to the above approach, will therefore depend, not only on the rate of cell-wall collisions, but also on the manner with which these collisions take place with a particular surface. The validity of this approach will be discussed in the light of the available literature reviewed below.

2.4.1 The Distribution of RBCs in Flowing Blood

The distribution of cells during the laminar flow of blood and various cell suspensions in capillary tubes has been studied by several workers (18,24,25,26,28). From their findings it has been suggested that, in the laminar flow of blood in a capillary tube, there exists a cell depleted layer near the tube wall known as the skimming layer. The factors influencing the thickness of this layer have been reported to include: the hematocrit, conduit size, flow rate, type of surface and on whether the flow is pulsatile or steady. Furthermore, the skimming layer, whose thickness is reported to fluctuate widely (24), does not seem to be completely cell free (24,25,26,27). A summary of these findings are presented in Table (2.1).

The RBC approximately resembles a thick disc of 8.5 μm in diameter and 2.5 μm in thickness. These dimensions are of the same order of magnitude as those reported for the thickness of the skimming layer. This, and the fact that cells have been observed, in nearly all cases, to collide with the wall, does seem
<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell suspension</th>
<th>Tube diameter</th>
<th>Hematocrit</th>
<th>Thickness of skimming layer:micrometre</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayliss (28)</td>
<td>defibrinated blood</td>
<td>100</td>
<td>40%</td>
<td>2-5</td>
<td>- cells observed to touch the tube wall.</td>
</tr>
<tr>
<td>Goldsmith (25)</td>
<td>RBC and ghost suspensions</td>
<td>75</td>
<td>0.01%</td>
<td>15</td>
<td>- frequent cell-wall collisions. (*)&amp;</td>
</tr>
<tr>
<td>Bugliarello (24)</td>
<td>Blood</td>
<td>40 - 83</td>
<td>30% (‡)</td>
<td>=100/hematocrit</td>
<td>- large standard deviation, with results independent of tube size and flow rates.</td>
</tr>
<tr>
<td>Phibbs &amp; Burton (26)</td>
<td>Blood in quickly frozen arterioles</td>
<td>1000</td>
<td>40%</td>
<td>2 - 6</td>
<td>- cells occasionally touching tube wall</td>
</tr>
</tbody>
</table>

(‡) Photographs show a marked reduction of the layer, roughly by a factor of 3, when blood velocity is reduced from 0.11 cm/sec. to 0.015 cm/sec.

(*) An additional element of uncertainty here is that, during flow, the RBC and ghost cells (empty membranes) undergo varying degrees of deformation. Goldsmith (3) suggests that, in capillary flow, the particle deformability is an important factor influencing the thickness of the skimming layer.
to throw considerable doubt on the concept of the cell-depleted layer. Of more importance, however, the above findings cannot, as implied by Blackshear (12,18), be justifiably extrapolated to blood flows in tubes of much larger diameters. This can be illustrated by the fact that blood flows in tubes, whose I,D. is less than 0.05 mm, is non-Newtonian, (27).

In the absence of further information, particularly on blood flows in large diameter tubing, it seems advisable to assume a uniform cell distribution throughout the crosssection of the tube's diameter.

2.4.2 Rate of RBC-Wall Collisions

Keller et al (29,30) have studied the flow of RBC suspensions in a narrow tube of 1 mm I.D., whose internal walls have been coated with radioactive cholesterol. The cholesterol, which is sparingly soluble in water, has been reported to enter and leave the membrane of a RBC with relative ease (30). Keller et al have shown the degree of cholesterol uptake, by the RBC suspending medium (Ringer-Lactate solution), to be very small. Furthermore, the degree of cholesterol uptake by the RBCs has been shown to be much larger than can be accounted for by a diffusional process. By assuming the amount of cholesterol taken up per wall collision to be constant, Keller et al have concluded that the radioactivity of the RBCs gives a relative measure of the overall rate of cell-wall collisions.

Keller's findings do show that the radioactivity of the cholesterol uptake by the flowing RBCs increases, approximately in a linear manner, with rising flow rates. This clearly
implies that the cell-wall collision rate is dependent on the Reynolds Number, (as based on the valid assumption of Newtonian blood flow within the tube). This in turn probably reflects a direct relation between the enhancement of the migration rate of the RBCs and the shear stress at the wall.

Keller's results also show that the cholesterol uptake of the cells increases disproportionately with rising hematocrit values of the flowing suspensions.

It is worth noting that the RBCs in the above experiments have been obtained from outdated human blood, which contains cell fragments and possibly ghosts (empty membranes). The above work seems to be based on the assumption that variations of hematocrit level and flow rate affect equally the transport of unruptured cells, ghosts and membrane fragments to the wall. The validity of this assumption may be questioned on the ground that the degree of deformability of a RBC may be an important factor when considering the rate at which it migrates towards the walls of a tube (25).

In their early works, Blackshear et al. (32) and Bernstein et al. (5) utilized a diffusion model, in an attempt to estimate the rate of cell-wall collisions, and the fraction of these collisions that result in cell rupture. They based their theory on the assumption that these collisions are mainly due to the diffusion of cells from the core of the tube to the walls. The suggested theory assumes the diffusion of cells to be analogous to the process of heat transfer in the entry region of a tube. Their conclusions suggest that the fraction of cells that hit the walls of a particular tube is constant, and independent of
the flow rate. These conclusions are highly suspect and contradict the experimental findings of Keller et al. (29, 30). The heat transfer analogy utilised can only hold if the cells are absorbed or removed by the tube walls on virtually every collision. Even then, it would only hold in the entry region. Furthermore, the fraction of cells ruptured by the collision processes will most likely depend both on the flow conditions and the type of surface involved.

What may be concluded from the insufficient literature, reviewed above, is simply that for a given tube surface, the rate of cell-wall collisions increases with rising hematocrit levels and blood flow rates. Clearly, more information is needed to determine the contribution of the fluid shear stress towards the enhancement of cell migration to the tube walls.

2.4.3 Adhesion of RBCs to Non-Biological Surfaces

The adhesion of the RBC to non-biological surfaces, has been reported by several workers (6, 7, 33, 34). Photographs of adherent cells have been produced, which reveal various ways by which a RBC may attach itself to a surface. Hochmuth et al. (6, 7) have observed that most of the cells attached to a raw glass surface appear to adhere along irregular outlines of the cell membrane. Those that adhere at a single point or along one regular line are rare. In the case of the flow of cell suspensions over fibrinogen, plasma and serum coated glass, the RBC has been observed to attach itself by a long strand, called a tether by Blackshear (23), with the bulk of the cell being lifted off the surface (6, 7). The maximum lengths of single tethers have been reported to vary from 24 μm (23) to 160 μm (18).
Photographs of adherent cells have been taken with a scanning electron microscope, SEM, at various angles between the electron beam and a normal to the surface (7). SEM photographs show how, after the flow of a cell suspension over the surface, the hemoglobin in the membrane of an adherent cell is pushed towards the down-stream edge of the cell, with the upstream edge appearing thin and relatively free of hemoglobin.

2.4.4 Effect of Wall Shear Stress on Adherent RBCs

Hochmuth et al (6) have examined the effects of low levels of wall shear stress, $\tau_w$, on adherent cells. Human RBCs are allowed to settle, by the action of gravity, on a raw glass surface, which constitutes the upper part of a parallel-plate flow channel. The adherent cells are then subjected to a determined level of $\tau_w$, by a controlled flow of a solution of buffered saline. At low stress values, $0 < \tau_w < 10$ dynes/cm², the cells have been observed to elongate increasingly with rising values of $\tau_w$. Significantly, Hochmuth et al mention that when the flow is stopped, i.e. $\tau_w = 0$, the cells are observed to return to a disc-like shape with a certain amount of permanent distortion at their upstream edge. Additional, but proportionately less elongation, is observed at $10 < \tau_w < 100$ dynes/cm²: the RBCs at 64 dynes/cm² have been observed to be 80% longer than the case for 7 dynes/cm². However, at such relatively high levels of stress ($10 < \tau_w < 100$ dynes/cm²) the cells have been reported to stay attached to the surface for only a short period of time ($\sim$ seconds) (6).

Hochmuth et al (7) have also investigated the magnitude of the shear stress required to detach an adherent RBC
from various surfaces. The RBCs, suspended in buffered saline solution, are allowed to settle by the action of gravity, on a particular surface for 10 minutes. The number of adherent cells, at various radii, are counted. The surface is then mounted on a rotating shaft, dipped in saline, and allowed to rotate at a constant angular velocity for a period of 5 minutes. After the run, the adherent cells, at various radii, are again counted. It is very likely that the saline fluid layers adjacent to the rotating disc will acquire a rotational motion. Hochmuth et al do not clarify how this fluid motion affects their results. Nevertheless, the results, shown in Table (2.2), indicate that there is a minimum value of wall shear stress, $\tau_{w \text{ min}}$, below which cells remain adherent to the surface. Above $\tau_{w \text{ min}}$, the fraction of detached cells has been found to increase, approximately in a proportional manner, with rising levels of $\tau_w$. For a given value of $\tau_w$ (above $\tau_{w \text{ min}}$), the fraction of detached cells has also been found to increase with longer time durations of the applied stress. This may probably reflect the viscoelastic nature of the RBC membrane.

<table>
<thead>
<tr>
<th>Surface</th>
<th>$\tau_{w \text{ min}}$ (dynes/cm²)</th>
<th>$f$</th>
<th>$\tau_{w \text{ max}}$ (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw glass</td>
<td>10.2</td>
<td>1.00</td>
<td>27</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>4.5</td>
<td>0.95</td>
<td>18</td>
</tr>
<tr>
<td>Silicone-coated glass</td>
<td>3.6</td>
<td>1.00</td>
<td>15</td>
</tr>
<tr>
<td>Teflon</td>
<td>2.5</td>
<td>0.80</td>
<td>8</td>
</tr>
<tr>
<td>Fibrinogen on raw glass</td>
<td>1.7</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Plasma on raw glass</td>
<td>1.7</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

$\tau_{w \text{ min}}$ = minimum wall shear stress required to initiate detachment,

$\tau_{w \text{ max}}$ = maximum wall shear stress which when sustained results in the detachment of all adherent cells,

$f$ = fraction of cells that remain adherent after $\tau_{w \text{ min}}$ has been applied.

Table (2.2)
After the above described shearing process, an examination of some of the adherent cells to the various surfaces, has revealed a trail of material, deposited on the surface and leading up to the point(s) at which the visible cells are attached (7). This has been interpreted as a track of the 'dragging' movement of the cell during the shear flow. Also, observations of the raw glass surface, made with an SEM, have revealed certain amount of 'debris', probably membrane material left behind by the detached cell. The above findings strongly suggest a form of sub-lethal damage of the adherent and detached cells.

Using the parallel-plate flow channel, Hochmuth et al (7) have also performed an experiment to determine if cells could contact, and subsequently adhere to a raw glass surface, under steady flow conditions. Initially, a steady flow of saline solution is established in the channel. RBCs suspended in saline (of 40% hematocrit) are then passed for a period of 25 minutes, followed by a saline solution alone. The adherent cells are then fixed by a flow of dilute gluteraldehyde solution. The same flow rate is maintained throughout the procedure. The fixed cells, all observed to be deformed in shape and aligned in the direction of flow, are counted. The results show the complete absence of adherent cells at \( \tau_w \) values greater than 10 dynes/cm\(^2\). At lower stress values, the number of adherent cells has been found to increase sharply with decreasing values of \( \tau_w \). In these experiments, SEM photographs of adherent cells which have been subjected to a wall shear stress of 5 dynes/cm\(^2\), for one hour, and then detached by a wall stress of 30 dynes/cm\(^2\), clearly show visibly altered cell shapes.
Blackshear (12,18,17) has proposed a model of the particular case of a cell attached to raw glass by a single tether, whereby the value of $\tau^*_w$ required to detach the tethered cell is estimated at 120 dynes/cm$^2$. This value is more than four times the reported experimental value for raw glass (7). The above mentioned model is based on the reported estimates of the tether dimensions, which as mentioned earlier, vary considerably. Furthermore, the model assumes that the shearing stress will detach the cell at the section where the tether joins the surface. Kochen (33) has shown that cells attached by single tethers may be caused to lyse, rather than detach, when subjected to low values of $\tau^*_w$ (estimated to be much less than the above value of 120 dynes/cm$^2$).

George et al (34) have examined the effect of fibrinogen, serum and plasma on the adhesiveness of RBCs to uncoated raw glass surfaces. Their findings indicate that the presence of these blood constituents, even in small concentrations ( ~ 1% by volume), in the cell suspending medium (saline), has a very marked decreasing effect on the adhesiveness of RBCs to raw glass surfaces. Fibrinogen and plasma seem to have equally diminishing effects on the adhesiveness of cells to raw glass. Serum, however, seems more favourable in promoting adhesion than plasma. This may indicate fibrinogen as the adhesion diminishing component of plasma. (This is opposite to the effect that fibrinogen seems to have on platelet adhesion to raw glass(35)). However, heating plasma to 56°C, thus precipitating the fibrinogen, does not seem to alter the adhesion diminishing properties of plasma. This may indicate other components of plasma as playing a role in the adhesion diminishing process.
George et al (34) have also examined the effects on cell adhesion to raw glass, due to various changes in the properties of the suspending solution, saline: the pH (6.1 - 7.5), solution's ionic strength (by a factor of two), and the presence of small concentrations of cationic proteins (1 mg/ml lysozyme, 0.1 mg/ml protamine, and 0.01 mg/ml poly-L-lysine). These changes have been found to have insignificant effects on the adhesiveness of RBCs to raw glass surfaces.

2.5 Discussion and Conclusions

In the absence of non-biological surfaces, the viscoelastic nature of the RBC membrane renders the process of cell rupture dependent on both the magnitude and the duration of the applied shearing stress. The reviewed literature suggests that RBCs hemolyse when exposed to levels of shear stress of the order $1.5 \times 10^3$ dynes/cm$^2$ for the relatively long time period of few minutes. It is probable that such high levels of stress may exist in regions of flow discontinuities, e.g. in the turbulent wake of an occlusive prosthetic valve. However, blood flows in extracorporeal and prosthetic devices is such that very few RBCs are likely to be entrained in flow discontinuity regions long enough ($\sim$ minutes), to sustain outright rupture. Nevertheless, it seems probable that in circulatory flows, successive exposures of the RBC to such stress levels, will induce some form of sub-lethal damage and ultimately, cell rupture.

In steady and pulsatile blood flows in large and smooth conduits, relatively low levels of shearing stress are
developed. For example: for laminar and turbulent flows of up to 7 litres/minute in a smooth tube of 1.3 cm I.D., the maximum developed level of shear stress (i.e. wall shear stress), is less than $10^2$ dynes/cm$^2$. These low stress levels are very probably sustained for the entire duration of the blood flow. When remote from non-biological surfaces, the contribution, if any, of these low stress levels to the RBC damage, is most likely to be confined to some form of sub-lethal damage.

For the flow of blood over non-biological surfaces, the reviewed literature is insufficient in providing information about the mechanisms of cell-surface interactions. However, the works of Hochmuth et al (6,7) and others (29,30,34), do give certain indications about the probable hemolytic roles played by the various levels of the wall shear stress. These are outlined below.

The rate at which RBCs collide with a given surface appears to increase with rising hematocrit levels and increasing blood flow rates. It is not clear what role, if any, the fluid shear stress plays in enhancing the migration of cells towards surfaces. However, the outcome of a cell-surface interaction will probably depend on both the nature of the surface and the wall shear stress level, $\tau_w$.

In flowing blood, the adhesion of RBCs to raw glass surfaces seems unlikely to occur at $\tau_w > 10$ dynes/cm$^2$. In comparison, cell adhesion to teflon, polyethylene and silicone-coated surfaces seems even less likely. Furthermore, the literature suggests that RBCs which adhere to, and are subsequently detached from surfaces, are likely to sustain a form of sub-lethal damage - as indicated by observed changes in their shape and size, as well-
as losses of some material from the membrane and its contents.

At $\w > 10$ dynes/cm$^2$, the mechanism and the hemolytic effects of cell-wall collisions, are unclear. It is probable that cells would collide with and be reflected away from surfaces. If as a result, a RBC sustains a form of sub-lethal damage during its brief encounter with the wall, then it would seem likely that successive collisions would ultimately result in cell rupture.

It is probable that at very high values of $\w$, the colliding RBC would rupture on impact with the surface. Instantaneous hemolysis has been estimated to occur when the pressure difference across the cell's membrane is $\sim 12,000$ dynes/cm$^2$, (i.e. $\sim 1.2 \times 10^{-2}$ atmospheres) (11). It has been suggested that this value may be achieved at wall shear stress levels of $\sim 10^4$ dynes/cm$^2$ (17,18).
CHAPTER THREE

A MOCK ARTERIAL CIRCUIT FOR PULSATILE BLOOD PUMPS

3.1 Introduction

In the body, the blood flow system is composed of two circulatory loops: the systemic and the pulmonary circuits. Each of these consists of an atrium, a ventricle and a system of arterial, capillary and venous beds. Much of what follows applies to both loops. Attention however, will be mainly confined to the systemic circulation.

As pulsatile blood pumps are designed to simulate the action of the ventricle, a proper evaluation of these pumps necessitates the construction of a mock arterial system which can reproduce the overall dynamic properties of the systemic physiological tree. This necessity arises from at least two considerations, both of which have bearing on the hemolytic studies to be undertaken. The first relates to whether the pump is capable of furnishing the required flow rates at physiologically tolerable pressures. The second concerns the proper functioning of the specific type of valve, the Edinburgh Valve, employed at both the pump's inlet and outlet positions.

The composite elements of the systemic circulatory loop are shown in Figure (3.1), together with the corresponding elements that have to be reproduced in a mock system.
Figure (3.1)  (a) Composite elements of the cardiovascular loop, (b) composite elements required in a model.
3.2 General Requirements of The Mock Circulation

The mock loop must in the first place have the haematological characteristics compatible with the pump's hemolytic testing programme. Specifically, the traumatic effects of the mock system should, under given conditions, be reproducible and of a magnitude that is small enough to allow for the detection of hemolysis produced by any of the factors under study. For if the hemolysis produced by the mock loop is very large, then the hemolytic contribution of any of the studied factors may be a small percentage of the total hemolysis, and variations produced by a change in any such factor may well be within the total margin of error.

The literature reviewed in Chapter (1) seems to indicate that the factors which contribute most to blood trauma are regions of high shear stress and the presence of non-biological surfaces. It therefore seems reasonable to assume that, in order to lower the hemolysis rate produced by the mock system, the following features are desirable:

(i) The smallest possible surface area exposed to blood; more specifically, the minimum attainable surface area / blood volume ratio.

(ii) All flow passages of the mock system should be of large cross-section, smooth and streamlined. This would ensure that, for a given flow rate, relatively low levels of shear stress are obtained within the blood stream, as well as at the non-biological surfaces.

(iii) Absence of regions and sites of fluid stagnation within the mock loop, ensuring a thorough mixing of the blood within
the system. It must be mentioned, however, that as yet there is no clear evidence in the literature relating hemolysis to blood stagnation. The above preference is stated, merely to attain a uniform hemolytic exposure of every element of the pumped blood.

The complexity of the physiological arterial tree and its intricate structure are too immense to reproduce in a model. This complexity is highlighted by the changes that take place on moving down the tree. These include changes in the size and compliance of arteries and their degree of branching, as well as changes in the complex wave velocity of the blood flowing through them (31). Furthermore, the Newtonian flow of blood in large arteries changes to non-Newtonian flow when blood is flowing through vessels whose internal diameters are less than 0.05 mm (27 ). All the above result in significant variations in the amplitude and contour of the pressure and the flow waves at different positions in the arterial tree (27 ).

The mock system called-for need not have all these characteristics, provided that the pressure and the flow waves at the inlet of the mock system are the same as those prevailing at the inlet of the ascending aorta of the physiological tree. In other words, the pulsatile pump should view the mock loop, as a whole, in exactly the same manner as the heart would view the entire arterial tree. This overall view, i.e. the 'black box' approach, greatly simplifies the design and construction of the desired model.

The conditions prevailing at the inlet of the ventricle which also have to be reproduced at the inlet of the
pulsatile pump, are relatively less complicated than those prevailing on the aortic side. The atrium simply acts as a low pressure reservoir, with a maximum pressure range of 5 – 10 mm Hg. Furthermore, it seems reasonable to assume that the pressure and the flow pulses generated at the ventricle's outlet, have completely died away before reaching the atrium. In a mock system the atrial reservoir should act as the end-point of a pressure/flow cycle and as a supplier of 'depulsed' blood for a following cycle. Any other arrangement may very likely result in the setting up of a form of resonance within the entire mock cardiovascular loop.

The significance of reproducing the aortic pressure-flow (P-F) relationship, in terms of magnitude and phase, may be emphasised in several respects:

(i) Dynamic evaluation of the Pulsatile Pump: When the pump is loaded with an impedance which is comparable to that of the systemic arterial tree, the pressure developed within the pump's chamber, as well as its flow output, may then be directly compared with the corresponding pressure and flow waves of the left ventricle. Such a comparison would indicate whether the required flow rates are being furnished at physiologically tolerable pressures.

The aortic P-F relationship can also provide a quantitative assessment of the power requirements to be met by an artificial heart. This power is determined from the sum of the kinetic and potential (i.e. pressure related) energies delivered by the ventricle (52).
(ii) Evaluation and improvement in the design of the Edinburgh Valve: Unlike other prosthetic valves, the ultimate design of the Edinburgh Valve is aimed at utilizing the flow of blood to operate the occluding vane throughout the pulse cycle. As yet the role which the pressure wave plays in the operation of the vane is unclear. There are indications, however, which strongly suggest that the pressure wave not only contributes to the initial opening of the vane, but also affects the degree to which it is maintained open during the flow cycle. It is as yet unclear how the vane of a certain valve design would be affected by reflected pressure waves and changes in the mean arterial pressure. It would seem reasonable, therefore, to assume that the proper assessment of any valve design does necessitate the faithful reproduction of the aortic flow and pressure waves.

The relevant features of the aortic P-F waves may be characterised by the input impedance of the systemic circulation. The input impedance, discussed in the following section, is expressed in terms of a modulus and a phase angle. The impedance spectrum may be represented in a diagram wherein the moduli and the phase angles are plotted against the frequency. The reproduction of the impedance spectrum of the systemic circulation in a model is, as explained below, sufficient to characterise the pressure and flow waves at the inlet of the model.
3.3 Vascular Resistance and Hydraulic Input Impedance of The Physiological Arterial Tree

For many years, physiologists have used the concept of vascular resistance when characterising the flow of blood in the various vascular beds of the arterial tree. The quantity is computed as the ratio of the mean pressure difference across the vascular bed to the average flow through it. Vascular, or peripheral, resistance finds its analogy in the d.c. electrical theory of flow. The term, however, is insufficient to describe the situation when the pulsatile nature of pressure and flow are to be taken into account.

Recently, especially with the advent of reliable flow meters of rapid response, physiologists have come to rely more on the concept of the hydraulic impedance, or vascular input impedance. This is defined as the ratio of the instantaneous pressure to the instantaneous flow, as measured at the same site, at the inlet of a vascular bed, (which for the whole arterial tree is the root of the ascending aorta). The input impedance term is used by analogy with the electrical a.c. theory.

The following treatment of input impedance is for sinusoidal waveforms. For these we have (see Figure (3.2)):

\[ p(t) = P_i \cos(\omega_i t + \phi_i) \]
\[ f(t) = F_i \cos(\omega_i t + \beta_i) \]

- \( p(t) \) = the pressure at any time \( t \),
- \( f(t) \) = the flow at any time \( t \),
- \( P_i \) = modulus of the pressure wave
- \( F_i \) = modulus of the flow wave
- \( \omega_i \) = frequency
- \( \phi_i \) = phase of pressure wave
- \( \beta_i \) = phase of flow wave
Figure 3.2 (a) Quantities characterising the sinusoidal pressure and flow signals needed to compute the impedance term. $P$ and $F$ are the amplitudes of the pressure and the flow waves respectively. $\phi$ and $\beta$ are the phase angles for the respective pressure and flow waves. (b) Complex representation of the pressure and the flow waves shown in (a). In both (a) and (b) the diagrams show the flow leading the pressure.
The impedance $Z$, at the frequency $i$, has an amplitude $Z$ and a phase angle $\phi$ given by:

$$Z = \frac{P}{F} \quad \text{and} \quad \phi = \varphi - \beta$$

A more elegant method of expressing $Z$ involves the use of complex numbers, thus:

$$p = P_1 \exp \left[ j \left( \omega_1 t + \phi_1 \right) \right] \quad \text{where} \quad p(t) = \text{real part of } p$$

$$f = F_1 \exp \left[ j \left( \omega_1 t + \beta_1 \right) \right] \quad \text{where} \quad f(t) = \text{real part of } f$$

$$Z = \frac{p}{f} = \left( \frac{P_1}{F_1} \right) \exp \left[ j \phi_1 \right] \quad \text{where} \quad \phi_1 = \varphi_1 - \beta_1$$

and $j = \sqrt{-1}$

As $Z$ is a complex number, it has real and imaginary parts:

- real part of $Z = \left( \frac{P_1}{F_1} \right) \cos \phi_1$
- imaginary part of $Z = \left( \frac{P_1}{F_1} \right) \sin \phi_1$

Again, by analogy with the electrical a.c. theory, the real part of $Z$ is its resistive component, and the imaginary part is its reactive component. It is worth recalling that in a steady, non-pulsatile, flow the pressure and the flow are in phase, as is the case when computing the peripheral resistance term.

As the resistive component of the impedance is always zero or positive, the angle $\phi_1$ must therefore lie between $+90^\circ$ and $-90^\circ$. When the angle $\phi_1$ is negative, the flow is said to lead the pressure. Then, by analogy to the electrical theory, the reactive component of the impedance can be shown to be predominantly capacitive. When the angle $\phi_1$ is positive, the pressure is said to lead the flow, and the reactive component of the impedance can be shown to be predominantly inductive.

$Z$, as calculated above, is determined from the pressure and the flow amplitudes at the particular frequency $i$. 
The calculation must be repeated for all frequencies of interest, and the impedance is displayed as a function of frequency, i.e. an impedance spectrum. The value at zero frequency (i.e. the d.c. value) is the peripheral resistance.

3.4 Harmonic Analysis

The measured wave forms of the pressure and the flow pulses in the arterial tree are not sinusoidal. As the impedance $Z_i$ is defined above for sinusoidal waveforms only, further computations are therefore necessary.

The decomposition of a pulse, of whatever form, into sinusoidal components is mathematically possible, provided certain conditions are fulfilled. The method employed, the Fourier Series method, is well known and practised in engineering. The details of the application of this method by numerical techniques are described in Appendix (A).

The conditions necessary for the application of the Fourier Series method to the observed pressure and flow pulses in the arterial tree merit close examination. These conditions state that:

1. The pressure and the flow pulses must be periodic: i.e. the pulses should have a constant frequency.

2. The arterial system must be linear. This implies that:
   (i) an imposed sinusoidal flow signal should produce one sinusoidal pressure signal only, and of the same frequency,
   (ii) when the amplitude of a sinusoidal flow wave is increased by a certain amount, the amplitude of the resulting pressure
wave should increase by the same relative amount, and with no changes in the phase,

(iii) when two or more sinusoidal flow pulses of different frequencies are imposed simultaneously on the system there should result independent sinusoidal pressure waves whose sum gives the observed pressure response.

Attinger et al. (36) have examined the variation in the heart rate of a number of anesthetised dogs. Their findings show that the variation in the observed rate over experimental periods ranging from 10 minutes to 4 hours, is small, such that the standard deviation from the average frequency is of the order of 3%. Furthermore, in order to obtain a representative cycle, they suggest that several cardiac cycles need to be sampled over several respiratory cycles.

Noble et al. (37) have analysed the aortic impedance spectrum of the dog when the frequency of the fundamental harmonic is changed and when the form of the input flow pulse is varied. Their findings show that the impedance moduli, (defined as $Z_i = P_i / F_i$) and the corresponding phase angles fall within a comparatively small band of the charted spectra, and conclude that the non-linearities in the arterial tree are very small. Bergel et al. (38) have reached the same conclusion during their experiments on the canine pulmonary artery, viz.: the impedance spectra are essentially independent of the heart rate, and the harmonic components of the impedance do not interact detectably. Dick et al. (39) have perfused the aorta of a dog with pump-generated sinusoidal flow waves and have found no significant distortions in the resultant sinusoidal pressure waves.
It seems reasonable to assume, therefore, that the non-linearities of the arterial tree are small, and do not invalidate the Fourier Series method.

3.5 Impedance Spectra of The Physiological Arterial Tree

The corresponding aortic pressure and flow spectra for man and dog are shown in Figure (3.3). These are computed from the pressure and wave recordings taken at the root of the ascending aorta. The values at zero frequency are equivalent to the mean pressure and mean flow waves. The most apparent feature of these spectra is the steep fall, with rising frequency, of both the pressure and the flow modulii. An implication of this is that the bulk of the pressure and flow waves are carried by the first few harmonics. More than 90% of the pressure and flow waves are contained within the first six harmonics, (this corresponds to a frequency of ~12 Hz for dogs and~7.5 Hz for man (42,43)). It therefore follows that the energy content, kinetic and potential, of harmonics beyond the 6th is small.

Figures (3.4) and (3.5) show typical results for the aortic input impedance spectra in both man and dog respectively. The d.c. values are the amplitudes at zero frequency and are equivalent to the peripheral resistances. Within the first few harmonics, the impedance spectra in both man and dog drop sharply from their high d.c. values. The spectra then remain steady, with relatively small fluctuations throughout the frequency range shown. Over this range, the mean of the impedance values is termed the characteristic impedance, and is found to be equivalent to the input impedance of the very large arteries close to the ventricle.
Figure (3.3) Aortic Pressure and Aortic Flow Spectra for Man and Dog.
Figure 3.4 Input impedance of the systemic arterial tree for Man. (The data is collected from several individuals).

Figure 3.5 Input impedance of the systemic arterial tree for Dog. (The data is collected from several dogs).
The characteristic impedance values for man and dog are less than 1/15th and 1/20th of their respective peripheral resistance values.

The fact that there are no large fluctuations in the impedance spectra indicates the absence of major reflection waves in the arterial tree (42). Wave reflection sites in the tree, however, are numberous and occur at every bifurcation and wherever there are changes in the viscoelastic properties of the vessels (27). The combined effects of these reflection sites seem to cancel each other, resulting in only minor fluctuations in the impedance spectra. In man, the first maximum in the spectrum occurs at ~7 Hz, with a magnitude of less than 1/6th of that of the peripheral resistance (42). O'Rourke et al (43) show that reflections in the arterial tree of the dog occur at ~5.5 Hz and ~10.5 Hz. These reflections they show are due to 'apparent' reflection regions, one in the top part, and the other in the lower part of the body.

The phase differences between the pressure and the flow waves, for the various harmonics, are also shown in Figures (3.4) and (3.5). The phase angle at zero frequency is, by definition, zero, i.e. the pressure and the flow are in phase, as is the case in steady flow. The angle $\psi$ is negative for the first few harmonics, and increases gradually until it becomes zero at ~4 Hz. At higher harmonics, the phase angle oscillates around zero. The initial negative phase indicates that the flow is leading the pressure. This is a characteristic feature of a predominantly capacitive system: i.e. the capacitive effects in the arterial tree dominate the inertial effects, over the first few harmonics.
In hypertension, arteriosclerosis and related
diseases, where there is a rise in the mean arterial pressure
and a reduction in the distensibility of the arteries, the
effects on the input impedance are very marked. Both the d.c.
and a.c. components of the impedance spectra are increased,
particularly at low frequencies, < 4 Hz, where the bulk of the
flow and pressure waves are carried. This results in the raising
of the entire spectrum, and its shift to the right. This implies
that the left ventricle would have to exert more power to
maintain a given flow rate (43,45,46).

The input Impedance of the systemic circulation
expresses the opposition which the left ventricle has to overcome
in order to drive blood through the systemic arterial tree. The
work required may be divided into two parts. The first is the
steady, or d.c. term, which arises from the energy dissipated in
driving the mean flow through the total peripheral resistance.
The d.c. component depends only on the dimensions of the vascular
system and on the viscosity of the blood. The second part is the
pulsatile component, or a.c. term, which arises from the energy
dissipated by oscillatory flow of blood e.g. in expanding the walls
of the arteries and overcoming the inertial of the flowing blood.
This a.c. term depends mainly on the viscoelastic properties of the
arteries and their variation down the tree, the architecture of
the entire tree (i.e. sizes of arteries and their degree of
branching) and to a lesser extent on the viscosity of blood (47,48).

As can be seen from Figures (3.4) and (3.5),
the design of the arterial system is such that the extra work
required by the ventricle to overcome the a.c. component of the
total impedance, is very small. This is estimated at between 5 to 15% of the total (46).

3.6 Synthesis of a Mock Arterial System

The foundation work for the modern theory of pulsatile fluid flow in rigid and elastic tubes has been laid down by Womersely (49). Since then, numerous theories have been put forward describing hydraulic, mechanical and electrical analogues to the arterial tree (50, 51, 52). The majority of these theories lay their emphasis on predicting the changes that occur to the pressure and flow waves, the viscoelastic properties of the vessel walls and the complex wave velocity, on moving down the arterial tree. Apart from the few exceptions, discussed below, none have attempted to set up a real hydraulic model of the entire tree.

The model introduced by Kolff (53) attempts to reproduce the mean atrial and mean aortic pressures by simply providing adequate hydraulic heads at the inlet and the outlet of the pump respectively. The model neither attempts to reproduce the relationship between the instantaneous aortic pressure and flow waves nor does it take into account the pulsatile flow of the fluid. Indeed, when the pulsatile pump shown in Figure (3.6) was loaded with such a model of the circulation, the outlet valve (Edinburgh type) was found to flutter irregularly with the flow or to remain open throughout the pulse cycle.

The hydraulic models adopted by the Hydrospace Company (54) and Westerhoff et al. (55, 56), are particularly designed to take into account the pulsatile nature of the flow.
Both models are based on the theoretical work of Womersely (49). But whereas the latter model takes the desired overall view of the arterial tree, the former attempts to reproduce a detailed substitute of the systemic circulation, relying on a host of pneumatic and electronic backing equipment, thus rendering it too complex to control and reproduce in a simple form.

The theoretical background to the above two models merits some explanation. Basically, the input impedance of the systemic circulation may be viewed as being the vector sum of its resistive, capacitive and inductive components. The resistive component of the input impedance consists of two parts. The first part is attributed to the viscous blood flow in the numerous small blood vessels, and is approximately equal to the peripheral resistance $R_p$. The second part, $R_c$, is associated with the viscous blood flow in the large and compliant arteries close to the ventricle. For a given frequency $i$, $R_c$ is given by: $R_c = Z_i \cos \varphi_i$ (where $Z_i$ is the characteristic impedance at frequency $i$, and $\varphi_i$ is the phase angle). For steady flow the absolute value of the input impedance is given by $|Z_i| = R_p + R_c$. At frequencies above ~3 Hz, the absolute value $|Z_i|$, is equivalent to $R_c$ only.

The capacitive component of the input impedance is attributed to the compliance of the aorta and the large arteries. In quantitative terms the capacitance of a system relates the rate of change in its volume to the rate of change in the pressure, i.e. Capacitance, $C = dV/dP$. The inductive component of the impedance is attributed to the inertia of the blood flowing in the arteries. It is dependent on the crosssectional area of each artery and the
density of the blood. Westerhof et al. (55) give an estimate of the capacitive and resistive components of the systemic input impedance for man and dog, Table (3.1).

<table>
<thead>
<tr>
<th></th>
<th>Man</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_p$</td>
<td>1,200</td>
<td>4,500</td>
</tr>
<tr>
<td>$R_c$</td>
<td>90</td>
<td>220</td>
</tr>
<tr>
<td>$C \times 10^6$</td>
<td>800</td>
<td>260</td>
</tr>
</tbody>
</table>

Table (3.1)

Each of the three elements of the input impedance may be independently reproduced in a model, such that when combined they reproduce the total input impedance of the systemic circulation. Thus the capacitor may be constructed from a compliant tube of known elastic properties, or from an air reservoir, (a bell-like chamber with an air pocket maintained above the fluid). The inductive and resistive components, on the other hand, may be constructed from rigid tubes. These tubes may be set in parallel form to achieve compactness. As both the inductance and the resistance are dependent on the diameter of the tubing, the construction of a pure resistor and a pure inductor is complicated however, and requires some theoretical analysis.

For the pulsatile Newtonian flow of blood in a rigid tube, of diameter $2r$, Womersely's theory (49) relates the resistive and the inductive components of the tube's impedance thus:

$$\frac{\text{Fluid Resistance}}{\text{Fluid Inductance}} = \frac{8F}{\cos \xi}$$
F is a function of the dimensionless ratio, \( \alpha \), and
\[
\alpha = r \sqrt{\frac{\omega}{\nu}}, \quad \nu = \text{kinematic viscosity of blood,} \quad \omega = 2 \pi f.
\]
The angle \((90° - \phi)\) is the phase difference between the pressure gradient and the flow, with \(\phi\) being a function of \(\alpha\).

The condition for \(\phi\) to approach \(90°\), whereby the pressure gradient is in phase with the flow is:
\[
6 \gg \alpha^2
\]
If the frequency of the highest harmonic of interest is \(\omega\), and taking blood as the flowing fluid \((\mu \approx 3 \times 10^{-2} \text{ poise}, \rho = 1.05 \text{ gm/cm}^3)\), then a purely resistive tube may be obtained if its radius, \(r\) (cm), is given by:
\[
0.828 \gg 2r(\sqrt{\omega})
\]
In other words, for a purely resistive tube, for which \(\phi = 90°\), the radius should be as small as possible, particularly at high frequencies. Thus if the highest harmonic of interest is 6 Hz, then the diameter of the purely resistive tubing should be much less than 3.4 mm.

The dimensions of the resistive tubing used in the Hydrospace and Westerhof models are given in Table (3.2), together with some calculated data for the case of blood when flowing at a steady flow rate of 5 litres/minute.

<table>
<thead>
<tr>
<th>Hydrospace Model</th>
<th>Westerhof Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{R_p + R_c}{p} )</td>
<td>( \frac{R_p}{p} )</td>
</tr>
<tr>
<td>Number of tubes</td>
<td>260</td>
</tr>
<tr>
<td>Diameter of tube</td>
<td>1.5 mm</td>
</tr>
<tr>
<td>Length of each tube</td>
<td>91 cm</td>
</tr>
<tr>
<td>Total Surface area exposed to blood</td>
<td>11,150 cm²</td>
</tr>
<tr>
<td>Surface/volume ratio</td>
<td>26.7 cm⁻¹</td>
</tr>
<tr>
<td>Maximum wall shear stress</td>
<td>95 dynes/cm²</td>
</tr>
</tbody>
</table>

Table (3.2)
3.7 The Adopted Model of The Systemic Circulation

For the proposed hemolytic studies, the adopted model of the circulation should be designed to produce the least possible trauma to the flowing blood. This overriding consideration does impose severe restrictions on the design and construction of the model. As mentioned above, the model may be constructed of three independent units, simulating the capacitive, inductive and resistive components of the desired input impedance. Whereas it is relatively simple to design the reactive components, the resistive component, however poses major hemolytic problems.

As stated earlier, blood pumping circuits should have the minimum surface area, specifically, the minimum surface/volume ratio, and for a given flow rate, the lowest possible levels of wall shear stress. The fact that a pure resistance must necessarily be constructed from tubing of very small diameter indicates that a system as that of Westerhof et al, though successful in reproducing the physiological impedance spectra, will nevertheless, be highly traumatic to the flowing blood. In order to obtain a model of relatively low traumatic characteristics, it is clear that tubes of much larger diameters would have to be employed. This, unavoidably, increases the inductive component of the circuit. In other words, in a model in which rigid tubes of large diameter are to be used, in order to reproduce the desired input impedance, there will be no independent control on the inductive and resistive components. Thus, taking blood trauma as being of paramount importance, it seems necessary to sacrifice any attempt to include a pure resistance in the mock circulation.
In Westerhof's model, the inverted bell-like air reservoir used to simulate the capacitive component of the impedance is easy to construct and can be controlled to produce the desired degree of compliance. Such a capacitor, however, harbours regions of fluid stagnation. Despite the absence of a clear indication in the literature relating blood stagnation to trauma, the elimination of these regions does result in a more uniform traumatic exposure of all elements of the flowing blood. A capacitor constructed from a compliant tube is therefore regarded as being more favourable than the air reservoir.

The adopted model of the circulation is also intended for use in the hemolytic assessments of the Edinburgh valve. It is essential that both the inlet and outlet valves operate satisfactorily and in the same manner as that intended when they are placed in a corresponding site in the heart. Satisfactory valve performance, for the Edinburgh type valve, entails:

(a) quick and uninterrupted opening of the vane to the maximum desired angle,
(b) absence of vane 'flutter' throughout the cycle,
(c) quick and uninterrupted closure of the vane, at the end of systole, and
(d) minimum of fluid regurgitation across the valve throughout the flow cycle.

Ideally, a pulsatile pump, of variable characteristics, when loaded with a circuit of the desired input impedance should provide an adequate test-rig for studying the performance of the valves employed. If, due to the use of blood as the working fluid, such a circuit is unattainable, then the adopted model should nevertheless ensure the reproduction of the desired valve's performance.
In the adopted model, the order of priorities has therefore been set thus: low traumatic characteristics and reproduction of the desired valve behaviour, followed by a reproduction of the input impedance of the systemic circulation.

The adopted model of the circulation is shown in Figures (3.6, 3.7). The blood volume of the mock system, including the Mk I pump and the atrial reservoir is 1250 ml. The total surface area exposed to blood is 1550 cm$^2$, and the surface/volume ratio is 1.2 cm$^{-1}$. The maximum wall shear stress, (excluding the stress developed in the vicinity of the valves) is 20 dynes/cm$^2$, (as based on the laminar flow rate of 5 litres/min.). For hemolytic considerations, these figures compare most favourably with those of either the Hydrospace or Westerhof models.

In the adopted model the capacitance of the system is provided by a piece of silastic rubber tubing, 25 cm in length, contained in a glass chamber which is kept open to the atmosphere. The inductive and resistive components of the model's impedance are attributed to two factors:

1. the pulsatile flow of the fluid within the silastic tubing,
2. the pulsatile flow of the fluid within the 1/2 inch tubing which connects the capacitor to the atrial reservoir. (This tubing is constructed from fairly rigid materials, PVC or silicone rubber).

When the pump is loaded with the model, the behaviour of the outlet valve has been found to depend on the following factors (most of which can be varied):

1. the proximity of the compliance to the valve: the closer the compliant tubing to the outlet valve, the more adequately
Figure (3.6) The mock cardiovascular loop. (a) Pulsatile pump, fitted with Mk I pump chamber (b) inlet valve housing, (c) outlet valve housing, (d) glass chamber containing the compliant silastic tubing, (e) atrial reservoir, (f) hydraulic tubes.
Figure (3.7): The Mock Cardiovascular Loop
The valve performs.

(2) The degree of compliance of the silastic rubber tubing: the bigger the capacitance of the silastic rubber chamber the better the performance of the valve. An indicative test of this feature is that when the atmospheric outlets of the glass container are closed, the outlet valve is found not to function at all, i.e. it remains open throughout the pulse cycle.

(3) The hydrodynamic design of the vane and the geometry of the flow passages in its immediate vicinity: in general, the cylindrical form of the Edinburgh Valve, Figure (5.7), has been found to perform more satisfactorily in the mock loop than the prosthetic form of the valve, Figure (5.6). The design of the ring of the prosthetic form has been found to have a very marked influence on the vane performance. This has been found to hold even for the most favourable hydrodynamic vane design.

(4) The adjustable parameters of the driving actuator: generally, the outlet valve behaviour has been found to improve with rising frequency, higher stroke volumes and shorter systolic times.

The effects on the behaviour of the outlet valve of varying the resistance and the inductance of the system are found to be negligible compared to those of changing the compliance of the circuit. Thus when a rigid tube, 2 inches in diameter, is attached to the pump outlet, the outlet valve is observed to behave in the same manner as when a 1/2 inch rigid tube is used.

The behaviour of the pump inlet valve has been found to be independent of the performance of the outlet
valve, and to be unaffected by any changes in the structure of the mock loop. Increase of atrial head (as measured by the height of the fluid within the atrial reservoir above the inlet valve) is found to increase the degree to which the vane of the valve opens. However, an increase in the atrial head necessarily increases the mean pressure in the entire system. A limit is reached when the compliant silastic tubing is fully extended within its glass chamber, thereby cutting down its capacitance considerably. Improvements in the performance of the inlet valve can therefore be achieved by either improving the vane design or by varying the geometry of the flow passage in the immediate vicinity of the valve, or both.

3.8 The Input Impedance of The Adopted Model

The input impedance of the mock circulation has been determined by analysing the pressure and the flow recordings taken in the vicinity of the outlet valve, on the downstream side. The pressure wave has been recorded using a hypodermic needle connected to a Statham transducer, as shown in Figure (3.8). The frequency response of the transducer is 100 Hz, which is well above the frequency range of interest, 0 - 12 Hz. This frequency range contains more than 95% of the pressure and the flow waves, and is adequate for comparison purposes with the reported impedance spectra of man and dog. The flow signal has been recorded using an Electromagnetic Flow Meter, EFM, of adequate frequency response (50 Hz). The cuff of the flow meter is specially designed for use in-vivo, where it is placed around
**Figure (3.8):** Measuring sites of the pressure and the flow waves.
the artery of interest. In order to preproduce a suitable measuring site in-vitro, a piece of freshly excised sheep aorta, 2 inches in length and 1/2 inch in internal diameter, has been inserted in the circuit, as shown in Figure (3.8). The compliance of the aorta has been found to be negligible when full compliance is maintained in the downstream silastic tubing. The effect of including the aorta and its connecting tubing must necessarily increase the inductive component of the total impedance, and to a lesser extent, the resistive component as well.

The pressure and the flow signals were recorded simultaneously on light sensitive chart paper, set at a speed of 44 cm/sec. The zero and the full range marks (200 mm Hg and 400 ml/sec.) were marked on the moving paper. The signals of three consecutive cycles were recorded. Each cycle was divided into 24 equal time intervals and the mean of the corresponding ordinates in the three cycles was taken. The variations in the magnitude of the corresponding ordinates are small in nearly all cases, with a maximum deviation of not more than 10% of the mean. The error in reading the ordinates from the recording paper is ± 0.5 mm, corresponding to ± 0.8 mm Hg and ± 1.8 ml/sec. This error will not affect the magnitude or phase of the computed impedance values, and they will only appear in the calculation of the mean pressure and the mean flow waves.

The error involved in digitising the signals into 24 intervals is calculated indirectly. The impedance values have been calculated for three cycles at three different frequencies, when each cycle is digitised at 24 and 96 intervals. A comparison
of the two sets of results shows that up to the 6th harmonic, the impedance values for the cycle with 24 intervals are within 7% of the corresponding values for the case of digitising at 96 intervals. For higher harmonics, the differences are not more than 30%. For harmonics at which wave reflections occur, ~6th, the error may be as high as 100%. These large errors are attributed to the very small magnitude of the flow modulii above the 6th harmonic. The smallest variations in these magnitudes are much amplified when the impedance term is computed.

Figure (3.9) shows the input impedance spectra for the model, at the three fundamental frequencies 36, 50 and 66 r.p.m. In a linear system, all the charted points of the spectra, of whatever fundamental frequency, should fall on the same lines. In the model, however, deviations from linearity increase sharply above 7.5 Hz, the probable resonant frequency of the silastic tubing. At lower frequencies, the relatively smaller deviations from linearity may be attributed to a host of likely sources: the compliance of the bellows of the driving actuator, the probable pressure dependence of the capacitance of the silastic tubing and the consequent non-linearity of its inductance, and the computational errors due to insufficient digitisation.

A comparison between Figure (3.9) and Figures (3.4) and (3.5) shows that the input impedance of the model is much higher than those of the arterial systems of either man or dog. The d.c. resistance of the model however, is of the same order of magnitude as that found in the human system.

In the model, the phase angle is initially positive,
Figure 3.9 Impedance modulii and phase angles of the three pairs of pressure and flow waves, shown on the right. Deviations from linearity increase sharply at frequencies above 8 Hz.

The first reflected wave occurs at \( \sim 4.5 \) Hz, as indicated by the very high magnitude of the impedance modulus at this frequency. The phase angle is initially positive, dropping to zero at \( \sim 2 \) Hz and becoming negative in the range 2 - 5.5 Hz.

The resistive and reactive components of the circuit are given in Appendix (E).
which indicates the pressure wave leading the flow wave. This is in direct contrast to the observed phase angles in both man and dog. At low frequencies, the inductive components of the impedance seem to dominate. This is not unexpected in a model which is constructed of large diameter conduits. The largest contributor to the inductance, seem to be the silastic tubing (~50 cm² in cross-sectional area when distended). When the cross-sectional area of the silastic tubing is decreased (by clamping the atmospheric outlets of the glass container) the inductance of the system is reduced. Thus despite the accompanied reduction in the compliance of the system, the phase angle now becomes initially negative, as shown in Figure (3.10(b)).

The presence of at least one large reflected wave in the model, occurring at ~4.5 Hz, is in direct contrast to the absence of major reflected waves in the systemic arterial trees of both man and dog. Reflected waves in the model may in part be attributed to the abrupt changes in the elastic properties of the conduits of the system.

Figure (3.11) shows the pressure and the flow spectra for the model. The flow spectrum is in good agreement with that of man, shown in Figure (3.3). This is probably due to the fact that both the pulsatile pump and the left ventricle are positive displacement pumps with very similar systolic/diastolic time ratios, 1/3. The pressure spectrum in Figure (3.11) is appreciably higher than the corresponding spectra in both man and dog. This accounts for the relatively high input impedance of the model.
Figure 3.10 Effects of varying the reactive components of the mock arterial tree:

(a) When the 1/4 inch tubing, connecting the compliant chamber to the atrial reservoir, is clamped there results an increase in the d.c. resistance of the mock tree.

(b) When the compliance of the silastic chamber is reduced, by clamping the atmospheric outlets, there results changes in the a.c. components of the input impedance.
Figure 3.11 The Pressure and Flow Spectra of the respective waves, shown on the right, and which were recorded at the downstream side of the outlet valve of the mock cardiovascular loop.
When the 1/2 inch I.D. tube which connects the silastic chamber to the atrial reservoir is clamped, only the d.c. component of the impedance is increased, as shown by Figure (3.10 a). The a.c. components remain unchanged. When the compliance of the system is reduced, by clamping the atmospheric outlets of the compliant chamber, the a.c. components of the impedance are increased. No accompanied change in the d.c. resistance is observed, Figure (3.10 b). These features of the model are in qualitative agreement with the characteristic features of Westerhof's model (55), and the systemic arterial trees of both man and dog. (43,45).

3.9 Conclusions

A model of the physiological circulation is essential for evaluating the hemolytic and dynamic properties of the pulsatile pump and its Edinburgh Valves. The study of the dynamic characteristics of these valves necessitates the faithful reproduction of the magnitude and relationship of the aortic pressure-flow waves, i.e. the input impedance of the systemic circulation.

The attainment of the dynamic properties of the systemic circulation in a model necessarily involves a very large surface area exposed to blood, a high surface/volume ratio and relatively high levels of wall shear stress: factors which contribute to blood trauma. In constructing the model, the order of priorities has therefore been chosen: thus (1) use of the model in hemolytic studies, (2) use of the model for dynamic studies.

The adopted model of the circulation, though
successful in producing adequate valve behaviour, fails to reproduce the dynamic properties of the systemic arterial tree. The input impedance of the model is appreciably higher than the corresponding systemic impedance. Furthermore, the model fails to reproduce the systemic phase relationship between the pressure and the flow waves. The hemolytic properties of the adopted model, however, compare most favourably with the other discussed models of the circulation.
CHAPTER FOUR

DEVELOPMENT OF EXPERIMENTAL PROCEDURES FOR BLOOD PUMPING STUDIES

4.1 Introduction

The fragility of the RBC membrane depends on the type of animal species used and can vary according to the state of health, exercise, age etc., of individuals within the same species. Ideally, hemolytic assessments of blood pumps are best carried out using fresh human blood drawn from a single individual. In this study, the minimum blood volume required for each experiment is 2.5 litres. As the maximum safe amount of blood that can be donated by a person is 1/2 litre, the pooling of human blood donated by various individuals is inevitable. Such a pooling may result in the mixing of RBCs of various degrees of membrane fragility. However, the supply of fresh human blood, even for hospital use, is scarce - an ironic situation, considering the extent of blood shed throughout this world of political turmoil. Outdated human blood, three weeks old or more, is however, easily accessible. In the following sections its disadvantages and the relative merits and disadvantages of blood of other species is discussed.

4.2 Outdated Human Blood (Storage time ≥ 21 days)

The mean life span of a human RBC circulating the body is 120 days. If this rate of cell ageing is not exceeded in the outdated blood, then the percentage of 'overaged' cells (i.e. ≥ 120 days) in the outdated batch is estimated at not less than 17% (i.e. 21 x 100/120). The mean age of all other cells in the
batch would also be higher than the corresponding mean age in fresh blood. Furthermore, the fact that in outdated blood RBCs are stagnating under non-physiological conditions of gas and biochemical exchanges may further contribute to the fragility of membranes of cells of all ages. Thus in outdated blood the above factors are expected to significantly contribute to the number of ruptured cells and cells with severely fragile membranes.

Experiments have been conducted to investigate the general features as well as the reproducibility of results obtained with outdated human blood. The citrated blood (100 ml ACD/400 ml blood), all of the same age (21 days) and type (Group A, Rh+) is supplied in 500 ml bottles. The bottles, which are gently stirred to resuspend the RBCs are pooled together in one container. Using the circuit shown in Figure (4.1), half of the pooled blood is pumped and periodically sampled. After few hours of pumping, the blood is discarded and the circuit is rinsed, three times with distilled water and once with Ringer-Lactate solution. The second half of the blood batch is then pumped under identical pumping conditions for an equal number of hours.

Figure (4.2) shows typical results obtained with outdated blood. As expected, the initial levels of hemolysis, \( \sim 200 \text{ mg}\% \), as well as the average hemolysis rates, \( \sim 250 \text{ mg}\%/\text{hr} \), in both pumping runs are appreciably high. Furthermore, the hemolysis rates in both runs are not uniform: in the first hour of pumping, the rates are higher than those of the subsequent pumping hours. Figure (4.2) also shows how the second pumping run always produces higher initial hemolysis values and higher average hemolysis rates. This discrepancy between the two runs cannot be attributed to the
increase in the age of cells in the second batch: this age increase is a very small fraction of the total blood age. (The hemolytic effects of RBC ageing is discussed in a later section). The pooling of blood, from various individuals, though of the same grouping and factor, does therefore seem to largely contribute to the above observed differences. This indicates that mechanical cell damage in both pumping runs is augmented by 'biochemical' cell damage whose rate is unlikely to be the same for different blood batches. It is probable that the above observed differences may be attributed to the fact that in the second pumping run the surfaces of the circuit have been contaminated by the blood from the first pumping run. This however, does not explain the increase in the initial hemolysis level of the second blood batch.

The above findings also seem to suggest that due to the presence of a large number of ruptured as well as severely damaged cells, the obtained results tend to exaggerate even the gentlest of the sources of mechanical trauma. Also, it is probable that the presence of a large number of membrane fragments in the pooled blood may contribute to the rate of mechanical damage, e.g. by probably increasing the rate at which cells collide with the non-biological surfaces. Furthermore, during the experiment, cells are subjected to further damage of a biochemical nature, whose rate is difficult to establish and reproduce. It therefore seems that in the study of the mechanical sources of hemolysis, the use of outdated human blood does introduce distorting factors which can prevent reasonable reproduction of results.
The blood volume of the pumping circuit, from which air is excluded, is 0.75 litres.

Figure (4.1):
Figure 4.2: Hemolytic results of the pumped outdated human blood.
4.3 Ox Blood

The advantage of using ox blood lies in the fact that a large volume, ~6 litres, of blood may be yielded by the single animal. The blood, obtained from the slaughter house, is collected in ACD bottles (100 ml ACD/400 ml blood). Using the circuit shown in Figure (4.1), the citrated blood is pumped for periods of up to 8 hours. Figure (4.3) shows typical results obtained from one of three conducted experiments on the blood of various oxen.

The results show a high level of initial hemolysis, ~ 150 mg%. Following an initial rise, the rate of hemolysis remains almost constant for most of the pumping period. After 7 to 8 hours of pumping the blood changes colour from red to blackish red, and on centrifuging, no separation of the RBCs is obtainable. This indicates the total and almost simultaneous hemolysis of all cells in the batch.

The results do suggest that the membrane of the RBCs of ox blood can sustain a considerable degree of sublethal damage for long pumping periods, without being ruptured. Beyond a certain level of sub-hemolytic damage all the cells seem to rupture simultaneously. The values of free plasma haemoglobin can only give a measure of the number of ruptured cells. In view of this adopted method of assessing cell damage, the use of ox blood does not seem to be of much value in our hemolytic studies. (However, the 'toughness' of the RBCs membranes render ox blood suitable for oxygenation study purposes.)
Figure 4.3: Hemolytic results of the pumped ox blood.
4.4 Slaughter House Sheep Blood

This is obtained by filling citrated bottles with blood, flowing out of the severed jugular and carotid vessels of the slaughtered animal. In all cases the collected blood has been found to be appreciably hemolysed, $\sim 250 \text{ mg}\%$. This may have been due to haematological reactions attributed to the unclinical method of collection and the sudden and profuse bleeding of the animal - a horrific and most unpleasant scene!

4.5 Fresh Greyhound and Sheep Blood

4.5.1 Exsanguination: The animal which has been previously fasted for 24 hours, is anaesthetised by the intravenous administration of Nembutal (0.5 ml/kg. wt.). Usually, 0.5 ml of mucous heparin (500 units) are administered to ease the bleeding process. The blood is drawn via a cannula (3 mm I.D.) inserted in the femoral artery for dogs and the carotid artery for sheep. The blood is collected into either heparinised bottles (4 units/1 ml of blood) or citrated bottles (1 volume ACD/4 volumes blood).

The hematocrit of the exsanguinated blood has usually been found to increase slightly with the bleeding process: e.g. for greyhounds,

<table>
<thead>
<tr>
<th>Bottle</th>
<th>1st</th>
<th>3rd</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit:</td>
<td>61%</td>
<td>63%</td>
<td>65%</td>
</tr>
</tbody>
</table>

Thus, all the bottles are pooled together prior to the start of each experiment.

The entire bleeding process is slowed down so as to last not less than 15 minutes. This is achieved by raising the
level of the collecting bottle with respect to the animal. The slowed process is thought to minimise any hemolysis which may result from profuse and sudden bleeding of the animal.

Throughout this study, the initial hemolysis in the collected batches of fresh blood (from 15 Sheep and 37 greyhounds) has been found to be small. In the great majority of cases the initial hemolysis is less than 7 mg%, and the highest recorded value has been 17.5 mg%. An examination of all results has revealed no apparent relationship between the initial hemolysis and the hemolysis rate. (In the five experiments carried out on outdated human blood, however, high initial hemolysis has been found to result in high hemolysis rates.)

In all experiments, the initial hemolysis in the second of two pumping runs, in which blood of the same animal is used, has been found to be higher than the corresponding value in the first run. This is attributed to the ageing of the RBCs in the idle blood batch of the second run (as discussed in a following section). In two isolated cases the initial hemolysis of the blood batches used in the second runs was found to be very high, ~100 mg%, which resulted in the abandonment of both experiments.

4.5.2 Heparinisation

In some experiments e.g. those carried out on haemodilution, the blood from the animal is collected in heparinised bottles (4 units/1 ml blood). This level of heparin has been found to be adequate for maintaining the blood clot-free for the entire duration of the experiment (~12 hours). The above heparin
level has also been found to have negligible effect on the hemolysis rate of the bottled blood. Table (4.1) shows that for both diluted and undiluted blood, the increase in free plasma haemoglobin is very small and as discussed later, this increase is due to the ageing of the RBCs. (Blood dilution is effected by the addition of Ringer-Lactate solution).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time after Exsanguination hr:min.</th>
<th>Free plasma haemoglobin mg%</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound A</td>
<td>2:00</td>
<td>3.5</td>
<td>65% (undiluted)</td>
</tr>
<tr>
<td></td>
<td>3:00</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:00</td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>Greyhound A</td>
<td>1:45</td>
<td>1.8</td>
<td>30% (diluted)</td>
</tr>
<tr>
<td></td>
<td>5:00</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:00</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Sheep A</td>
<td>1:00</td>
<td>3.3</td>
<td>40% (undiluted)</td>
</tr>
<tr>
<td></td>
<td>4:00</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7:30</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Sheep A</td>
<td>1:00</td>
<td>2.0</td>
<td>20% (diluted)</td>
</tr>
<tr>
<td></td>
<td>4:00</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:30</td>
<td>5.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table (4.1)**

With outdated human blood, however, excessive heparinisation, 6 units/ml, has been found to induce some hemolysis in the blood batch.

One disadvantage of the use of heparin as an anticoagulant is that its activity decreases with time. When melted down, frozen samples of plasma have been found to contain a small amount of clot. Wherever possible, acid-citrate-dextrose solution has been used as the anticoagulating agent.
The minimum blood volume that is required for conducting an experiment consisting of two pumping runs in the system used here is 2.5 litres. The average volumes of undiluted blood that can be obtained from an exsanguinated animal are 1.5 and 2.0 litres for sheep and greyhound respectively. An account of the amounts of undiluted blood obtained from the 15 sheep and 37 greyhounds used in this study are given below:

Number of dogs:  2  9  3  7  5  6  2  3  
Blood Volume:  1.25  1.50  1.75  2.00  2.25  2.50  2.75  3.00 litres

Number of sheep:  2  3  4  1  2  3  
Blood Volume:  1.00  1.25  1.50  1.75  2.00  2.25 litres

It is clear that in order to obtain 2.5 litres of blood of the same animal, some dilution may be necessary. This has been effected by the addition of a sterile solution of Ringer-Lactate solution (Hartman's Solution).

It is probable that the addition of various volumes of lactate solution may induce some immediate or latent changes in the pH of the diluted blood and probably also cause some hemolysis. As sudden and large changes in the pH of blood are known to cause hemolysis the results of measurements made to assess the effect of the addition of various volumes of the lactate solution are shown in Table (4.2). These measurements indicate that the buffering capacity of blood is such that there is no significant change in the pH value that can be attributed to the addition of the lactate diluent. The small decrease in the pH values and the
small rise in the free plasma haemoglobin concentrations may be attributed to the increase in the time lapse between the exsanguination of the animal and the measurements. This time factor, which also affects the hemolysis rate of pumped blood, is discussed in a later section.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Volume Units</th>
<th>pH</th>
<th>Free plasma Haemoglobin</th>
<th>Hematocrit</th>
<th>Time hr:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep A</td>
<td>4</td>
<td>4</td>
<td>≤6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>7.44</td>
<td>2.3 mg%</td>
<td>38%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.40</td>
<td>6.8</td>
<td></td>
<td>5.30</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.43</td>
<td>3.7</td>
<td>19%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.39</td>
<td>4.9</td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>Sheep B</td>
<td>4</td>
<td>4</td>
<td>7.43</td>
<td>1.2</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.40</td>
<td>4.3</td>
<td>37%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.42</td>
<td>0.5</td>
<td>19%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.38</td>
<td>4.0</td>
<td></td>
<td>5.45</td>
</tr>
<tr>
<td>Greyhound A</td>
<td>4</td>
<td>4</td>
<td>7.33</td>
<td>6.1</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.30</td>
<td>8.5</td>
<td>63%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7.32</td>
<td>5.5</td>
<td>32%</td>
<td>6.00</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7.30</td>
<td>4.3</td>
<td>25%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7.29</td>
<td>6.1</td>
<td></td>
<td>5.45</td>
</tr>
<tr>
<td>Greyhound B</td>
<td>4</td>
<td>4</td>
<td>7.26</td>
<td>4.0</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.23</td>
<td>5.9</td>
<td>65%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.25</td>
<td>3.5</td>
<td>33%</td>
<td>5.30</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7.21</td>
<td>4.8</td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7.25</td>
<td>3.0</td>
<td>26%</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>7.22</td>
<td>4.0</td>
<td></td>
<td>5.15</td>
</tr>
</tbody>
</table>

Table (4.2)

The dilution of blood, however, has a direct bearing on the hematocrit level. The maximum recorded hematocrit of undiluted sheep and greyhound blood are 45% and 65% respectively. Dilutions in some cases may have to be carried out to the extent of lowering the final hematocrit to 20%. It seems reasonable to assume that small changes in the hematocrit may result in proportional changes in the
hemolysis rates of the pumped blood (all else maintained constant).
It is probable, however, that large deviations from the hematocrit of
the undiluted blood, e.g. 65% to 20%, may result in large and
disproportionate changes in the hemolysis rate, thereby magnifying
any hemolytic differences in the traumatic factors to be studies.
Although blood of the same animal and hematocrit value is used for
comparing a pair of traumatic factors, comparisons between various
experiments will have to take account of the hematocrit level of
each experiment. To investigate this experimentally, the exsanguinated
blood of the same animal, was divided into two or three volumes.
One undiluted volume was pumped and its hemolysis rate was compared
to those of the other diluted volumes which were pumped under
identical conditions. The circuit used is shown in Figure ( 4.1 ).
The partial occlusion on the roller pump was maintained constant
in all experiments. New PVC or silicone rubber tubing and new
compliant silastic rubber chambers were used for every experiment.
The blood volume of the circuit, from which air had been excluded,
was 0.75 litres. For the set occlusion, the roller pump was rotated
at 140 r.p.m. giving a flow rate of 4.8 litres/minute. In all
experiments the undiluted blood was pumped first. At the end of
the pumping period, the blood was discarded and the circuit was
rinsed three times with water and once with lactate solution.
Fresh blood of the same animal was then diluted to the required
hematocrit and pumped for an equal period of time.

The results for sheep and greyhound blood are
shown in Figures ( 4.4 ) and ( 4.5 ) and Table ( 4.3 ). The
slopes of the straight lines fitted to the data by the use of the
Least Squares method and the standard deviations of these slopes
are shown. For sheep blood the tabulated results do seem to suggest that the hemolysis rate is directly proportional to the hematocrit level. As the hematocrit of sheep blood rarely exceeds 45% this relationship may be assumed to apply to all dilutions. For greyhound blood however, increases in the hematocrit level do result in highly disproportionate increases in the hemolysis rate. A probable explanation of the above is that a rise in the cell density of the blood will markedly increase the rate of all collision processes, including the cell-surface collision rate. It is very likely that the collisions between the RBCs and the surfaces of the circuit are a major source of hemolysis, another being the flow conditions at the partially occluded section of the tube. Table (4.3) shows the RBC of the sheep to be less fragile than that of a greyhound (based on the assumption of equal cell density for both blood types). Thus an increase in the cell-wall collision rate seems more likely to affect the apparently more fragile cells of greyhound blood.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hematocrit</th>
<th>Hemolysis rate</th>
<th>Rate / % Hct.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg% / minute</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>s_b</td>
</tr>
<tr>
<td>Sheep A</td>
<td>17%</td>
<td>0.051</td>
<td>0.003</td>
</tr>
<tr>
<td>Sheep B</td>
<td>18%</td>
<td>0.093</td>
<td>0.009</td>
</tr>
<tr>
<td>Sheep C</td>
<td>20%</td>
<td>0.091</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>38%</td>
<td>0.188</td>
<td>0.005</td>
</tr>
<tr>
<td>Greyhound A</td>
<td>20%</td>
<td>0.254</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>0.914</td>
<td>0.093</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>2.015</td>
<td>0.084</td>
</tr>
<tr>
<td>Greyhound B</td>
<td>36%</td>
<td>0.329</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>2.098</td>
<td>0.160</td>
</tr>
</tbody>
</table>

b = hemolysis rate, mg% / minute,
s_b = standard deviation in b, mg% / minute.

Table (4.3)
Figure (4.4):  The hemolytic effect of varying the hematocrit of the pumped blood.

(The calculated slopes of the lines shown above are given in Table (4.3))
Figure (4.5): The hemolytic effect of varying the hematocrit of the pumped blood.
The above results are insufficient for providing a numerical relationship between the hematocrit level and the hemolysis rate. Nevertheless, they do demonstrate that when comparing two experiments conducted with greyhound blood, but of varying hematocrit levels, the hemolysis rate results of the blood of the higher hematocrit are very likely to be amplified.

4.6 Sampling

In the pumping circuit shown in Figure (4.1), samples are withdrawn via a hypodermic needle inserted into the midstream of the 1/2 inch tubing. In the circuit shown in Figure (3.7), the samples are withdrawn via a catheter (1.5 mm I.D. and ~7 cm long) attached to a syringe. The tip of the catheter is dipped to a position near the centre of the atrium before the sample is withdrawn.

The needle, the catheter and syringe (a new set is used for every experiment) are all thoroughly rinsed with Ringer-Lactate solution prior to the extraction of each sample.

To minimise any hemolysis that may be attributed to the sampling process, samples are extracted very slowly and gently such that a 6 ml sample is withdrawn in a period of ~1 minute. The resultant flows in the hypodermic needle and the catheter are laminar.

Samples, in duplicate, are withdrawn from the blood batch prior to the priming of the circuit. Two samples are usually withdrawn in the first hour of pumping, followed by a sample at every subsequent hour and a further sample at the end
of the pumping run. The volume of each sample is 6 ml, required to furnish the minimum of 2.5 ml of plasma needed for the free plasma haemoglobin measurement. The maximum total volume of all extracted samples is 72 ml (based on 6 duplicate sample pairs). Excluding the first and last pairs, the sampling volume is only 6.4% of the total pumped volume. As the same sampling procedure is applied to each of two pumping runs of any experiment, a relative comparison of the results will not be significantly affected.

The method devised for measuring the free plasma haemoglobin concentration of a sample is described in Appendix (B). The differences between the measured values in the duplicate samples have been found to be small. Most differences have been well within \( 2\% \), \( \% = \) known measurement errors). The mean of the duplicate pair is recorded. For some pairs, less than 10 in all, the measured values have been found to differ by more than \( 2\% \), with the highest recorded difference being 10.5 mg%. For these, the lower of the two readings is recorded. Such high differences are most likely to be the result of hemolysis occurring during the extraction of the sample. The known errors in the determination of the free plasma haemoglobin are those due to the measuring errors. These are estimated at not more than 5.5% of the measured value, (see Appendix (B)).

4.7 Presentation of Results

In an experiment, blood is pumped for periods of up to two or three hours. During the pumping period, samples are withdrawn at various intervals, and the free plasma haemoglobin
concentration, in mg%, is plotted on the y-axis with the pumping time on the x-axis. Figures (4.4 ) and ( 4.5 ) show typical results whereby the hemolysis clearly appears to be a function of pumping time. A visual inspection of all the graphs in this study strongly suggest that hemolysis bears a linear relationship to time: i.e. for the adopted pumping period, the hemolysis rate is constant. The fitting of a straight line to the experimental points on a graph does imply the theoretical assumption that for the given pumping duration the number of cells actually ruptured is a measure of the total damage sustained by the RBCs.

The choice of the pumping duration is dictated by two considerations: (1) that it should be long enough to expose any hemolytic differences between the factors under study, and (2) because of ageing factor (see following section) the duration should be short enough to allow for two pumping runs to be conducted within (say) 12 hours of the exsanguination process. Thus whereas the experimental results strongly suggest a constant hemolysis rate for the 3 hour period, this may not be the case for longer pumping durations.

The results of experiments with outdated human blood and ox blood do show the hemolysis rates in both cases to be non-linear. The apparent linear relationship that exists for fresh sheep and greyhound blood may not hold for fresh human blood: the effects of the initial hemolysis, autohemolysis, pumping duration and other factors that may influence the hemolysis rate of fresh human blood need to be examined.

To avoid the implication of linearity, therefore, it has been decided to present the results in a graphical form
in which the experimental points are joined by straight lines. However, as quantitative comparisons need to be made between the various results, the slope of the straight line that best fits the experimental points of each run is calculated.

The method of Least Squares has been applied to fit straight lines to the obtained data. The assumptions underlying this method are, that:

(1) errors in the x values are negligible,

(2) errors in the y values are normally distributed with constant variance, and

(3) errors in successive measurements are independent.

The maximum error in the determination of pumping time (i.e. the x values) is ±1 minute. This constitutes a maximum error of ±5%, ±1.7% and ±0.9% for the second, third and subsequent points respectively. In most cases a minimum of 6 points are obtained. Thus, with the exception of the second and third points the errors may be regarded as very small.

The maximum measurement error in the determination of the free haemoglobin concentration of a sample is ±3.5%. As only two measurements are taken for each point on the graph, these errors are assumed to be distributed normally around the mean (taken to be the mean of the two measurements). The variance for each point is also assumed constant.

Although not all of the above conditions are satisfactorily met, nevertheless, the Least Squares method is more consistent and reliable than the visual method of fitting straight lines to data. To fit a line of the form $a + bx$, the free haemoglobin concentration value of the ith point, sampled
after $x_i$ minutes of pumping time, is represented by $y_i$. The Least Square method (61, 62) then gives:

$$b = \left[ n \sum x_i y_i - \sum x_i \sum y_i \right] / \left[ n \sum x_i^2 - \sum x_i \sum x_i \right]$$

$$a = \left[ \sum y_i - b \sum x_i \right] / n$$

where $n = \text{number of experimental points}$.

For the calculated slope $b$, the standard deviation $s_b$ is given by:

$$s_b = \sqrt{s^2 / \left[ \sum x_i^4 - \left( \sum x_i^3 \right)^2 / n \right]}$$

where

$$s^2 = \frac{1}{n-2} \left( \sum y_i^2 - \left( \frac{\sum y_i}{n} \right)^2 \right) - b \left( \sum x_i y_i - \frac{\sum x_i \sum y_i}{n} \right)$$

For each set of data, the 'strength' of the fitted line needs to be examined, i.e. an examination of the likelihood of the line fitting the data by chance alone as opposed to the probability of there being a real relationship between $x$ and $y$.

For the slope $a + bx$, the correlation coefficient, $r$, is given by:

$$r = b \sqrt{\frac{n \sum x_i^2 - \sum x_i \sum x_i}{\sqrt{n \sum y_i^2 - \sum y_i \sum y_i}}}$$

For a positive slope, values of $r$ lie between 0 and 1. When $r=1$, the line passes through all the points and is thus a perfect fit. When $r=0$, no linear relationship is said to exist between $x$ and $y$. The smallest $r$ value for any of the lines fitted in this study is 0.95, which indicates a very good fit of the lines. This may be illustrated further by calculating the probability of the line fitting the data by chance alone. For a $t$-test, $t$ is given by:

$$t = r \sqrt{\frac{n - 2}{1 - r^2}}$$

For $n > 5$, $r > 0.95$ then $t > 5.5$. From the statistical tables, for $n - 2$ degrees of freedom, the probability of obtaining a fit by chance alone is given at less than 1%.
The results for each pumping run therefore, are presented in the following form:

\[ a + b t + s_b \]

where 
- \( a \): calculated initial hemolysis, mg%
- \( b \): calculated hemolysis rate, mg% / minute,
- \( t \): pumping time, minutes,
- \( s_b \): standard deviation in the hemolysis rate, mg% / minute.

### 4.8 Effect of Ageing on the Fragility of the RBC

Ideally, hemolytic comparisons should be carried out simultaneously in two separate loops, identical in every respect except for the factors to be examined. Because only one driving actuator mechanism is available (designed and constructed in this department), blood pumping runs are necessarily carried out consecutively. In the early experiments that have been conducted in this study, the hemolysis rate of the second of two consecutive runs, in which blood of the same animal is used, has been found to be higher than that of the first run, irrespective of the factor under study. This may be explained by either or both of the following: (1) the surfaces of the circuit may have been 'contaminated' by the pumped blood of the first run, and (2) the RBCs of the blood in the second run have 'aged' prematurely, thus rendering them more fragile. This ageing factor may be attributed to many probable causes: the exsanguination process, the stagnation of the cells while the blood is awaiting pumping, and the absence of gas and biochemical exchanges across the RBCs membranes during their stagnation.
To investigate the ageing process, two identical cylindrical jars A and B, of siliconised surfaces, are filled with equal volumes of blood, 225 ml, of the same greyhound. The surface/volume ratio of each of the blood filled jars is unity. This is comparable to the corresponding ratio of the circuit used for hemolytic studies (Figure (3.7)), 1.2 cm\(^{-1}\). The first jar, A, is gently rotated, at 70 r.p.m., and periodically sampled for a period of three hours. At the end of this period jar B, (in which blood has been kept under stagnant conditions similar to those experienced by the blood awaiting the second of two consecutive pumping experiments), is then rotated at the same speed and periodically sampled for an equal period of 3 hours. Some typical results are shown in Table (4.4).

<table>
<thead>
<tr>
<th>Greyhound</th>
<th>Hematocrit</th>
<th>Jar A</th>
<th>Jar B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30%</td>
<td>0.6 + 0.003 t, 0.000</td>
<td>1.2 + 0.019 t, 0.003</td>
</tr>
<tr>
<td>2</td>
<td>41%</td>
<td>0.3 + 0.014 t, 0.002</td>
<td>3.2 + 0.020 t, 0.004</td>
</tr>
<tr>
<td>3</td>
<td>46%</td>
<td>3.7 + 0.020 t, 0.006</td>
<td>10.4 + 0.050 t, 0.005</td>
</tr>
<tr>
<td>4</td>
<td>50%</td>
<td>6.2 + 0.006 t, 0.000</td>
<td>7.3 + 0.010 t, 0.000</td>
</tr>
<tr>
<td>5</td>
<td>50%</td>
<td>1.1 + 0.019 t, 0.002</td>
<td>6.1 + 0.022 t, 0.002</td>
</tr>
<tr>
<td>6</td>
<td>50%</td>
<td>-0.1 + 0.039 t, 0.002</td>
<td>1.1 + 0.079 t, 0.001</td>
</tr>
</tbody>
</table>

a = calculated initial hemolysis, mg%,
b = calculated hemolysis rate, mg% / minute,
t = pumping time, minutes,
s_b = standard deviation in b, mg% / minute.

Table (4.4)
Table (4.4) shows how jar B always produces a higher hemolysis rate than jar A. The differences in the two hemolysis rates usually rise with rising hematocrit levels, but can also vary from one greyhound to the next. As the two jars have identical surfaces, and the flow patterns in both are identical, and no contamination of the surfaces of jar B with 'used' blood from jar A occurs prior to its rotation, it can be assumed that the observed differences are due to the ageing of the RBCs in jar B, i.e. the time lapse between the end of the exsanguination process and the start of the pumping experiment.

This ageing of the RBCs does contribute to the hemolysis rate for every pumping run in the circuit. Alternating the factors under study in consecutive experiments is therefore advisable. An attempt to account for the ageing of the RBCs in an experiment may be obtained as follows: simultaneously with each pumping run in the circuit, a jar of a corresponding surface/volume ratio is filled with the same blood, and is gently rotated as described above. The hemolysis rate in the time-jar is then subtracted from the corresponding hemolysis rate produced by the circuit. This has been carried out in many of the experiments described in the next chapter. However, this method can only give an approximate correction to account for the ageing effect. For it cannot be claimed that the hemolytic contributions of the ageing factor will be equal in both the circuit and the time-jar. The hemolysis rates shown in Table (4.4) are but a small fraction of the hemolysis rates produced in the pumping circuit. An attempt has been made to increase the hemolysis rate in the time-jar, by introducing two hollow tubes inside the jar, thus increasing the
surface area exposed to blood. The results (greyhounds 2 and 3 of Table (4.4)) show no significant increase in the hemolysis rate. To multiply the hemolysis rate in the time-jar by a constant factor before subtracting the resultant rate from the hemolysis rate produced by the circuit would still not improve the above approximation: this constant factor would have to be arbitrarily chosen and would have to vary from one greyhound blood to the next.

The above findings do impose some limitations on the sensitivity of the adopted procedure when detecting hemolytic differences between any two factors under study. To alternate the study factors in consecutive experiments is therefore essential: e.g. in one experiment, using blood from greyhound G1, factor A is examined in the first pumping run and factor B in the second run; in a second experiment using blood from greyhound G2, factor B is examined in the first run and factor A in the second run. Hemolytic differences between any two study factors will therefore only be exposed if these differences are large enough to overcome the additional hemolysis due to the ageing factor.

4.9 Siliconisation

The circuit used in this study, Figure (3.7), is assembled from components made of different materials. The tubing is made from either silicone rubber or PVC and the compliant chamber is made of silastic rubber. The atrial reservoir is made of glass and the pump chamber is machined out of perspex. A new set of tubing and silastic rubber are used in each experiment. The surfaces of the atrial reservoir and the pump chamber, however, would have to be freshly coated with a suitable material for each
experiment. The most widely used coating material for blood pumping equipment are silicone compounds. The water repellency of these compounds have rendered them particularly useful for coating non-biological surfaces exposed to blood.

The property of water repellency of silicone stems from its ability to bind itself to surfaces. When the fluid is baked on the surface the residual hydroxyl groups of the silicone may condense with similar groups on the glass, (or hydrogen bonding may come into play between the hydroxyls on the surface of the solid and siloxane bondings in the silicone). This orients the siloxane part of the molecule so as to expose the organic and water repellent part of the molecule (e.g. the alkyl group -R)\(^6\).

\[
\begin{align*}
\text{alkyl group} & \quad - \text{Si-} \\
& \quad \quad R \\
\text{hydroxyl group} & \quad \quad - \text{Si-O-Si-0-Si-O-} \\
& \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad R \\
& \quad \quad \quad \quad \text{OH} \\
& \quad \quad \quad \quad \text{OH}
\end{align*}
\]

The usual method for siliconising glass is to prepare a 5% solution of silicone (MS 1107\(^*\)), dissolved in a suitable solvent e.g. acetone or ethyl alcohol. The glass which has been thoroughly cleaned, is then dipped in the solution and baked for 5 hours in an oven at a temperature of 140\(^\circ\)C. Using this method, it has been found that some of the silicone coating on the glass is eluted away after an 8 hour exposure to flowing blood. Furthermore, this method is unsuitable for siliconising perspex, a material that distorts when subjected to temperatures higher than about 50\(^\circ\)C. (N.B. As acetone etches perspex surfaces, its use for preparing the silicone solution is not recommended).

\* Silicone fluid, MS 1107, manufactured by Hopkins & Williams, Chadwell Heath, Essex, U.K.
A different method for the siliconisation of surfaces has been adopted. This method utilises the ability of silicone compounds, under certain conditions, to form long chain polymers which can then be coated on any surface. A silicoloid fluid (Silicoloid 201, ICI\textsuperscript{R}) is dissolved in a suitable solvent, o-xylene or toluene: 1 volume of silicoloid/4 volumes of solvent. Few drops of a curing agent (curing agent 'A', ICI\textsuperscript{R}) which causes the silicoloid to polymerise are added. The resultant solution is vigorously stirred and poured or sprayed onto the cleaned and dried surface, and allowed to drain. The surface is then placed in an oven set at 40°C, for a period of 15 hours. This long period ensures that all the solvent has evaporated from the surfaces, leaving behind a uniform, smooth and non-wettable coating of silicone rubber. To remove the coating, the surfaces are soaked in benzene or toluene for few hours after which the silicone layer peels off the surface.

To test the hemolytic effects of the silicone coating, experiments have been conducted whereby the coated surfaces, i.e. atrial reservoir and pump chamber of circuit shown in Figure (3.6), are used in one pumping run and identical uncoated surfaces in another. In both runs blood of the same greyhound has been used. The results, shown in Table (4.5) and Figures (4.6) and (4.7), suggest that the hemolytic differences are mainly those which are attributed to the time factor (RBC ageing factor), and any hemolysis that may be attributed to the silicone polymer is small. Whereas the results do not show the silicone to have any significant hemolytic advantages, its use throughout this study has nevertheless, been adopted so as to provide consistent, fresh and non-wettable surfaces for the atrial reservoir and the pump chamber.

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\# The help of Dr. D.N. Kapur is gratefully acknowledged.
<table>
<thead>
<tr>
<th>Greyhound Experiment</th>
<th>Hematocrit</th>
<th>Circuit with Siliconised surfaces</th>
<th>Time after exsanguination</th>
<th>Circuit with no Siliconised surfaces</th>
<th>% change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a + b x t, s_b</td>
<td></td>
<td>a + b x t, s_b</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>38%</td>
<td>1b 2.8 + 0.388 t, 0.008</td>
<td>4.5 hr</td>
<td>1a 1.0 + 0.246 t, 0.006</td>
<td>0.388 - 0.246 x 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45%</td>
<td>2a 4.5 + 0.469 t, 0.025</td>
<td>1 hr</td>
<td>2b 10.9 + 0.624 t, 0.039</td>
<td>-33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.5 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20%</td>
<td>3b 0.3 + 0.296 t, 0.002</td>
<td>4.5 hr</td>
<td>3a -0.3 + 0.136 t, 0.002</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45%</td>
<td>4b 1.3 + 0.330 t, 0.010</td>
<td>4.5 hr</td>
<td>4a -1.2 + 0.235 t, 0.007</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>47%</td>
<td>5b 9.4 + 0.245 t, 0.006</td>
<td>1 hr</td>
<td>5a 1.1 + 0.270 t, 0.009</td>
<td>-10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.5 hr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Blood Flow Rate = 3.3 litres/minute.

a = calculated initial hemolysis, b = calculated hemolysis rate, s_b = standard deviation in b, mg% / minute

(**) These refer to graphs shown in Figures (4.6) and (4.7).

Table (4.5)
**Figure (4.6):** Hemolytic effects of siliconised surfaces; runs with siliconised surfaces: 1b, 2a, 3b. (see Table 4.5 for further detail)
Figure (4.7): Hemolytic effects of siliconised surfaces; runs with siliconised surfaces: 4b, 5b.
(see Table 4.5 for further detail)
4.10  **Hemolytic Effect of Air-Blood Contact**

In the circuit used for conducting hemolytic comparisons, Figure (3.6), the blood is directly in contact with air in the atrial reservoir. Experiments have been conducted to hemolytically compare two reservoirs (1) one in which air is in contact with blood, as shown in Figure (3.6), and (2) a reservoir in which air has been excluded. The exclusion of air has been effected by the insertion, in the reservoir, of a balloon-like silastic rubber surface, as illustrated in Figure (4.8). During diastole the balloon is allowed to expand without stretching, so as to avoid negative pressures. During systole the balloon collapses as the reservoir is filled with blood.

The results of experiments in which the above two factors have been compared are shown in Figures (4.9) and (4.10) and Table (4.6). These show that the addition of the silastic rubber surface (~115 cm²) causes more hemolysis than would be obtained with air-blood contact alone. As the blood volume is the same in each of the two runs of any experiment, the above strongly suggests that the hemolysis produced by the circuit is dependent on the surface/volume ratio of the circuit. It is also possible that the silastic rubber material is hemolytically unsuitable, even when exposed to the gentle blood flow within the atrial reservoir. To minimise the total hemolysis produced by the circuit therefore, the air-blood contact was retained for all the subsequent experiments.

![Diagram](image-url)
<table>
<thead>
<tr>
<th>Greyhound Experiment</th>
<th>Hematocrit</th>
<th>Graph **</th>
<th>Air present</th>
<th>Time after exsanguination</th>
<th>Graph **</th>
<th>Air excluded</th>
<th>% change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42%</td>
<td>1b</td>
<td>a + b x t, ( s_b )</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>1a</td>
<td>3.1+0.476 t, 0.037</td>
</tr>
<tr>
<td>2</td>
<td>45%</td>
<td>2a</td>
<td>0.9+0.336 t, 0.007</td>
<td>1 hr</td>
<td>4.5 hr</td>
<td>2b</td>
<td>1.5+0.430 t, 0.023</td>
</tr>
<tr>
<td>3</td>
<td>40%</td>
<td>3b</td>
<td>0.7+0.307 t, 0.013</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>3a</td>
<td>11.1+0.338 t, 0.007</td>
</tr>
<tr>
<td>4</td>
<td>43%</td>
<td>4b</td>
<td>3.1+0.344 t, 0.014</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>4a</td>
<td>-1.0+0.597 t, 0.006</td>
</tr>
<tr>
<td>5</td>
<td>48%</td>
<td>5a</td>
<td>2.0+0.304 t, 0.025</td>
<td>1.0 hr</td>
<td>4.5 hr</td>
<td>5b</td>
<td>1.5+0.699 t, 0.049</td>
</tr>
</tbody>
</table>

\( a = \text{calculated initial hemolysis, mg\%} \)

\( b = \text{calculated hemolysis rate, mg\%/minute} \)

\( s_b = \text{standard deviation in b, mg\%/minute} \)

\( t = \text{pumping time, minutes} \)

** These refer to graphs shown in Figures (4.9 ) and (4.10 ).

Table ( 4.6 )
Hemolytic effect of blood-air contact;
Pumping runs in which contact was allowed: 3b, 4b, 5a.
(see Table 4.6)
Hemolytic effect of blood-air contact; pumping runs in which contact was allowed: 1b, 2a. (see Table 4.6)
CHAPTER FIVE

HEMOLYTIC COMPARISONS BETWEEN VARIOUS DESIGNS AND MATERIALS OF VALVES AND CHAMBERS OF A PULSATILE BLOOD PUMP

5.1 Introduction

The aim of this study is to investigate the main sources of hemolysis in a positive displacement pulsatile pump. The literature reviewed earlier seems to suggest that both the material of construction and the pattern of fluid flow within the pump chamber may have marked traumatic effects on the pumped blood. The intention has therefore been to compare the hemolytic effects of varying (i) the material of construction of the pump and its valves, (ii) the pattern of fluid flow within the pump chamber, and (iii) the design of the adopted valve, the Edinburgh Valve.

5.2 Designs and Materials

5.2.1 The Positive Displacement Pulsatile Pump

The pulsatile pump, its valves and the pump's driving mechanism have all been designed and constructed in this department. Basically, the pump consists of two chambers, the hydraulic and the blood chambers, separated by a flexible diaphragm. The hydraulic side, filled with a non-compressible hydraulic fluid, is connected to a driving actuator via two rigid tubes, of equal lengths, (Figure (5.1)). Essentially, the actuator drives the hydraulic fluid, water, whose motion is transmitted, via the flexible diaphragm, to the pumped fluid in the blood chamber. The use of a non-compressible hydraulic fluid ensures that the
The pumping assembly, (a) electric motor for driving the actuator, (b) the actuator, (c) blood filled time-jar, (d) blood filled mock cardiovascular loop.
diaphragm is positively displaced, in a pre-determined manner, irrespective of the fluid impedance encountered on the blood side of the flow. This is in contrast to the use of compressible fluids in pulse generators, where the diaphragm motion is dependent on the pressure of the driving gas. The detailed mechanism of the driving actuator, shown in Figure (5.2), has been fully described by the designer, Macleod (64), and copies of the relevant literature are found in Appendix (C). The variable parameters of the driving actuator, viz. systolic/diastolic time ratio (1/3 - 3), stroke volume (0 - 100 ml), and frequency (0 - 120 r.p.m.), may be independently preset, or varied during the flow. Furthermore, by controlling the volume of water in the bellows of the actuator, the end-systolic volume of the blood chamber (i.e. ventricular dead-space) may be controlled. This factor may affect the degree of blood stasis within the pump and the interaction between the diaphragm and the walls of the blood chamber. In practice, the usable range of the variable parameters is limited by the onset of resonances and disturbances within the entire mock cardiovascular system, e.g. irregular behaviour in the performances of the outlet valve, the bellows of the actuator, the aortic chamber and the diaphragm. These disturbances are probably due to the finite compliance of the bellows of the actuator and of the specific circuit with which the pump is loaded. The upper working frequency has therefore been confined to 90 r.p.m. In hemolytic experiments, the pumping frequency has been set at 70 r.p.m.

A disadvantage of positive displacement pumps is that during diastole, they may develop negative pressures (< 760 mm Hg) within the pump's blood chamber. Exploratory
The Actuator employed for driving the incompressible hydraulic fluid, water:
(a) water filled rubber bellows, (b) systolic/diastolic time ratio control, (c) stroke volume control.
experiments have therefore been conducted to examine the probable traumatic effects of negative pressures on the RBC. Using the circuit shown in Figure (4.1), in which a roller pump is used, it has been found that the hemolysis rate of the pumped blood increases with decreasing pressures of the air contained in the glass chamber, viz. three equal blood volumes, 0.75 litres each, at 20% hematocrit, when pumped at a flow rate of 4.8 litres/minute give the following:

<table>
<thead>
<tr>
<th>Pumping run</th>
<th>Animal</th>
<th>Air pressure in the glass chamber (mm Hg)</th>
<th>Hemolysis rate (mg% / hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>Sheep A</td>
<td>635</td>
<td>7.4</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>Sheep A</td>
<td>700</td>
<td>5.6</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>Sheep A</td>
<td>760</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The few data, shown above, indicate that RBCs may hemolyse when subjected to pressures below 760 mm Hg.

The possibility of introducing negative pressures within the pump chamber has been greatly reduced by introducing the following features:
(a) the atrial reservoir is rendered compliant by the introduction of a free surface, exposed to atmospheric pressure, and (b) the free surface of the atrial reservoir is maintained at a level of ~ 20 cm, above the pump's inlet valve. Furthermore, the design of the actuator mechanism is such that the diaphragm is positively driven only during systole.

Thus, in the conducted hemolytic studies, the observed changes in the atrial head, during a complete cycle, have been found not to exceed ~ 3 cm.
5.2.2 Blood Chamber

Three different designs of the pump's blood chamber have been constructed. The first of these, referred to as Mk I, is a machined block of perspex, mounted in a brass casing (Figure (5.3)). The perspex chamber consists of a spherical cap, 10 cm in diameter and 1.8 cm in height. The flow outlet is positioned at the centre of the curved surface. The inlet to the chamber is set at a small angle to the base (i.e. the neutral position of the diaphragm), and tangential to the chamber's curved surface. This inlet configuration results in the initiation of a vortex-like flow of blood during diastole, which persists throughout the systolic part of the cycle. When the ventricular dead-space is maintained above zero, the vortex has been observed to persist throughout, from one cycle to the next.

The cylindrical form of the Edinburgh valve, described below, is mounted in a nylon housing attached, by metal screws, to the brass casing, (Figure (5.3)). This arrangement applies to both the inlet and the outlet valves. As shown, the entire valve housing is such that the diameter of the inlet and outlet conduits of the blood chamber remain smooth and uniform up to the pivots of the vane. Beyond the pivots, the diameter of the valve perspex housing increases gradually from 1.5 cm to 1.8 cm. This diameter increase is unavoidable, and is due to factors inherent in the valve design.

The other two blood chambers are conical. Their designs, which are identical in every respect except for the configuration of their inlet conduits, are shown in Figure (5.4). The diameter of the base of each chamber is 10 cm and the diameters
Figure (5.3): The Mk I blood pump chamber, in its metal support. The inlet valve housing (not shown) is identical to the shown outlet valve housing. The dotted line N denotes the neutral position of the diaphragm.
Radial Dracula Pump Chamber

Tangential Dracula Pump Chamber

Figure (5.4)
of the inlet and outlet conduits are 1.5 cm. As in the Mk I chamber, the inlet of the first cone or funnel is set at a small angle to the diaphragm and tangential to the funnel's curved surface. This configuration also results in a similar vortex-like flow pattern within the chamber, which, due to the permanent and large end-systolic volume, is sustained throughout the flow. The inlet of the second funnel is also positioned at a small angle to the diaphragm, but set radially to the chamber's curved surface. As a result, the flow in the chamber is non-uniform, with a considerable degree of mixing of the blood within. These two chamber designs are affectionately referred to as Tangential Dracula (i.e. T-Dracula) and Radial Dracula (R-Dracula) respectively.

The inlet and outlet valves are secured to the Dracula chambers by means of pieces of silicone rubber tubing, as illustrated in Figure (5.5). This arrangement is sufficiently rigid to maintain a continuous straight conduit of nearly constant diameter.

Under identical conditions of flow rate etc., the vortex-like flow of blood in the T-Dracula chamber produces higher blood velocities, parallel to the chamber's surface, than would be obtained in the R-Dracula chamber. This difference results in higher levels of wall shear stress being attained at the surfaces of the T-Dracula chamber. The literature reviewed in Chapter (2), suggests that such differences are hemolytically significant. Experiments have therefore been conducted to compare the two chambers hemolytically.

The residence time of the flowing blood in the T-Dracula chamber has been found to be slightly longer than the
Figure (5.5): Adopted arrangement for connecting the inlet and outlet valves to the Dracula chamber.
corresponding time in the R-Dracula chamber. In an open pumping circuit, a red dye has been introduced into the T-Dracula chamber, under the following flow conditions: flow rate - 1.0 litres/min., systolic/diastolic time ratio - 1/3, frequency - 20 r.p.m., stroke volume - 50 ml. The red dye in the chamber has been found to completely disappear after ~6 cycles. Under the same flow conditions, the dye introduced into the R-Dracula chamber has been found to disappear after ~5 cycles. This result seems surprising, particularly since the swirl induced by the vortex-like flow in the T-Dracula had been expected to reduce the spread of residence time in the chamber. The above result may be explained by the following observations. During the fluid flow, the dye in the T-Dracula has been found to persist longest in a relatively thin column of fluid stretching from the centre of the diaphragm to the chamber's outlet. This has been confirmed by the fact that when small air bubbles are introduced into the chamber, the region where the bubbles are trapped longest is within the above mentioned fluid column. In the R-Dracula chamber, however, the introduced dye and air bubbles have been found to be uniformly distributed.

As indicated above, the vortex-like flows in both the Mk I and the T-Dracula chambers result in the attainment of relatively high values of wall shear stress. Nevertheless, it was expected that differences between the flows in the two chambers might be hemolytically significant. Matters may be so arranged that at the end of systole the flexible diaphragm is just in contact with the chamber's curved surface. This arrangement, which results in almost zero ventricular dead-space, may be achieved by sufficiently filling the bellows of the driving actuator. In the T-Dracula chamber,
the ventricular dead-space is relatively large, ~200 ml, and the diaphragm, as a result, does not come into contact with the inner surfaces of the chamber. Clearly, the blood residence time in the T-Dracula chamber is then much longer than the corresponding time in the Mk I chamber. A hemolytic comparison between these two chambers would thus help to reveal the traumatic role, if any, of blood stasis in the pump chamber.

In the hemolytic studies conducted here, the Mk I, R-Dracula and T-Dracula chambers were freshly siliconised prior to the start of each experiment. The 2 mm thick flexible diaphragm, made of deproteinated natural rubber, was also siliconised.

5.2.3 The Edinburgh Valve

The Edinburgh valve has been designed by Macleod (57). The details of the valve design are described in the relevant literature in Appendix (C).

Two forms of this valve have been developed: one suitable for prosthetic implantation, Figure (5.6), and the other specifically adapted for use in a pulsatile pump, Figure (5.7). The valves used in this study were of the latter design. In this form, the valve housing consists of a hollow perspex cylinder, part of the bore of which has been machined conical, Figure (5.8). A vane is held by two pivots, fixed to the walls of the housing and about which it can turn freely inside the cylinder.

In the study of the effects of changes in the valve design on RBC damage, two identical perspex cylinders or housings were fitted, one with a flat vane and the other with a hydrodynamically
shaped vane, Figure (5.8). The main difference in the mechanical
behaviour between these two vanes lies in the degree to which each
opens during systole. Figure (5.8), shows how the flat vane, when
fully open, extends to not more than 60° to the line perpendicular
to the axis of flow. This is observed to result in the creation of
turbulent flow regions, on the downstream side, close to the vane's
surface, Figure (5.9). In the hydrodynamic vane, due to lift forces,
the vane opens to a much larger degree during systole, 80°. As a
result, relatively very little turbulence in the vicinity of the
vane is observed.

The above described patterns of flow across the two
vane types, were observed during blood flows across the outlet valve
of the R-Dracula chamber. The conditions of flow were: flow rate -
3.3 litres/min., systolic/diastolic time ratio - 1/3, stroke volume -
50 ml, frequency - 66 r.p.m. When strong light was shone on the
transparent conduits, the flowing blood appeared to have relatively
dense lines or 'streaks' which followed the local direction of the
flow. This property of the flowing blood seemed to persist at all
velocities of the fluid.

The differences in the flow patterns across the above
two vanes, may be increased by further restricting the angle to
which the flat vane opens during systole. This was achieved by
inserting a delrin peg (an acetal resin), on the downstream side of
the vane, which prevents the flat vane from opening fully. A valve
whose vane is thus restricted is shown in Figure (5.8). The
downstream regions of fluid turbulence produced during systole,
and which are more severe than the corresponding turbulence in the
other two vane designs, are shown in Figure (5.9).
Figure (5.6) The pyrolytic carbon prosthetic form of the Edinburgh Valve, fitted with a hydrodynamically shaped vane.
Figure (5.6) The prosthetic form of the Edinburgh Valve, fitted with a flat vane. The entire valve, shown above, is constructed from stainless steel.
Figure (5-7) The cylindrical forms of the Edinburgh Valve fitted with (a) flat pyrolytic carbon vane of reduced angle of opening, (b) flat delrin vane and (c) hydrodynamic pyrolytic carbon vane. The fluid flow is from right to left.
Figure (5.8): The cylindrical form of the Edinburgh Valve fitted with various types of vanes, showing the angle to which each opens during systole.
The observed blood flow patterns across the various vanes of the cylindrical form of the Edinburgh Valve.
The adopted materials of construction of the vanes of the Edinburgh valve are delrin and pyrolytic carbon (discussed below). The accurate fitting of vanes, made from these materials, to the perspex housing is crucial for the proper working of the valve. These pivots should be accurately machined to fit into the precise dimensions of the grooves of the vane, and should also form a tight seal with the perspex wall. As mentioned below, a pyrolytic carbon pivot cannot be machined once the pyrolyte layer has been deposited. As a result, it has been found difficult to fit them satisfactorily to the perspex cylinder. Delrin pivots, on the other hand, may be accurately machined to fit tightly into the perspex walls, and have therefore been used throughout this study.

The 'pivot grooves' of the pyrolytic carbon vanes used in this study have been specifically designed for the prosthetic form of the valve. The designed opening angle of these vanes are therefore specific to the design of the ring of the prosthetic valve. This angle differs from the corresponding angle in the cylindrical valve. The use of these specific carbon vanes has been found to result in them opening beyond the central axis of the cylinder. In this position, the blood back flow, in late systole and early diastole, would not be sufficient to close the vane. To prevent this, a delrin peg, fixed to the perspex wall, is introduced into the downstream side of the vane, Figures(5.7), (5.8). The position of the peg is such that it will allow the pyrolytic carbon vane to open to the same degree as that of an identically shaped delrin vane. The peg, which is positioned well into the rear of the vane, is unlikely to contribute much to any fluid turbulence that may develop in this region, during systole. The use of pegs in
cylindrical valves, fitted with delrin vanes, was not necessary; the delrin vanes of these valves were specifically designed for the cylindrical form of the valve.

5.2.4 Materials of Construction

The materials chosen for the intended hemolytic investigations, are Delrin and Pyrolytic Carbon. Both of these materials have been separately used in the construction of the tilting occluder of the Bjork-Shiley prosthetic valve (59). It is understood that the use of Delrin, a rigid material made of an acetyl resin, has recently been stopped, for reasons which have no bearing on the material's biocompatibility or its thrombogenic and traumatic characteristics. It is claimed that "its propensity to absorb moisture during steam autoclaving may lead to temporary, irregular valve function, if proper drying instructions are not followed" (59).

Pyrolytic carbon has also been used in the construction of the occluding ball in the ball-and-cage valve type prosthesis (60). Essentially, the material is prepared by depositing a uniform layer of carbon, few microns thick, on a suitably shaped graphite substrate, in a fluidized bed reactor, at a temperature not far below 1500 °C (60). The structure and properties of the deposited carbon can be varied by controlling the temperature of the reactor. The deposited carbon may be rendered impermeable by sufficiently polishing the pyrolytic surface. The pyrolytic carbon has been used to construct various designs of the Edinburgh valve. The material has been kindly prepared by the Atomic Energy Authority, Springfields Works, Lancashire, U.K. The final polish of the carbon surface has been carried out in this department.
Another form of carbon, vitreous carbon, has recently been found to be suitable for use in the construction of prosthetic valves (64). Essentially, vitreous carbon valves may be obtained by making an enlarged model of the desired shape, out of a hydrocarbon resin. This is then baked in a suitable reactor, under controlled conditions of temperature and pressure. The baking process 'evaporates' the hydrogen atoms from the resin, leaving behind a vitreous carbon form of reduced size (by about 25%). The vitreous carbon surface is polished prior to use. The thrombogenic properties of this material have been recently investigated (65), and found to be equally favourable to those of pyrolytic carbon. The mechanical properties of vitreous carbon, however, renders it very suitable for the construction of the Edinburgh valve. In our hemolytic study, the original intention included the use of vitreous carbon in constructing the pump chamber and its Edinburgh valves. However, due to technical delays, the manufacturers could not supply the valves and chambers in time, before the conclusion of this work.

In conclusion, therefore, only delrin and pyrolytic carbon have been used in constructing the Edinburgh valves. Variations in the material of construction of the pump had to be abandoned.
5.3 Results of The Hemolytic Experiments

5.3.1 A Summary of the Adopted Procedure

The details of the adopted procedure are given in Chapter (4). As mentioned, the pooled blood, of the same animal, was divided into two equal volumes. Each volume was diluted, with Ringer-Laetate solution, to obtain the required amount for the pumping run.

The procedure for comparing two factors, which may be referred to as A and B, was as follows. The circuit, fitted with factor A was primed with the first volume of the diluted blood, and pumped for a period of 3 hours. During the pumping period, samples were withdrawn, centrifuged and their plasma fraction extracted. At the end of the pumping period, the blood was discarded and the circuit was fitted with factor B. The circuit was then thoroughly rinsed (3 times with distilled water and once with lactate solution).

The second volume of the diluted blood was then pumped and periodically sampled for 3 hours. In a following experiment, using blood from a different animal, the circuit was fitted with factor B for the first run, and with factor A for the second run. As the ageing factor is a major contributor to the observed hemolysis the time lapse between the exsanguination of the animal and the start of the pumping for each run is recorded.

The Mk I pump chamber was used in the hemolytic study of all vane designs and materials. In the first pumping run, the pump was fitted with a pair of identical valves, for the inlet and outlet positions. In the second run, an identical pair of valves, of a different design or material, was fitted in place of the first pair. In a following experiment, the two pairs of valves were
alternated. In these experiments, the ventricular dead-space in the Mk I chamber was maintained at \( \sim 15 \text{ ml} \).

In most of the experiments described below a volume of blood in a siliconised time-jar, was simultaneously rotated with each pumping run and periodically sampled. The volume of the jar was 240 ml, and its total surface area was 225 cm\(^2\). The jar was usually filled with 225 ml of blood, thus giving a surface/volume ratio of one cm\(^{-1}\). The corresponding ratio for the pumping circuit is 1.2 cm\(^{-1}\).

5.3.2 Hemolytic Results

I. Flat and Hydrodynamically Shaped Delrin Vanes

These vanes have been hemolytically compared under the following conditions:

<table>
<thead>
<tr>
<th>Pump Chamber</th>
<th>Mk I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumped Volume</td>
<td>900 ml</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>3.3 litres/minute</td>
</tr>
<tr>
<td>Frequency</td>
<td>66 r.p.m.</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td>50 ml</td>
</tr>
<tr>
<td>Pumping Period</td>
<td>3 hours</td>
</tr>
<tr>
<td>Systolic/Diastolic time ratio</td>
<td>1/3</td>
</tr>
</tbody>
</table>

As the material of construction of these vanes was delrin, pegs restricting the opening angle were not used in either valve design.

Simultaneously with each blood pumping run in the circuit, a siliconised time-jar containing 225 ml of blood of the same animal, was rotated (Figure (5.1)) and sampled.

The results of the hemolytic comparisons between
the flat and the hydrodynamically shaped delrin vanes are shown in Figures (5.10), (5.11) and Table (5.1). With the exception of experiment 5, all the results indicate that the flat vane is more hemolytic to the flowing RBCs, than the hydrodynamic vane. This suggests that the relatively more severe turbulent regions in the vicinity of the flat delrin vane are a major source of RBC trauma. In experiment 5, it is likely that some hemolytic factor has been accidentally introduced in the 5a pumping run. For despite the fact that this run has been pumped first in experiment 5, its hemolysis rate is nevertheless, higher than any of the other listed runs.

As Table (5.1) shows, the results would be qualitatively unchanged, if the hemolysis rates in the time-jars are excluded. For as mentioned in the previous chapter, the trauma produced by these jars is very small compared to the trauma produced by the circuit.

II. Flat and Hydrodynamically Shaped Pyrolytic Carbon Vanes

The pyrolytic carbon vanes of these valves are fitted with delrin pivots. In the hydrodynamically shaped vane, a delrin peg is positioned on the downstream side of the pivots, so as to allow the vane to open to the same degree as that of an identically shaped delrin vane.

To increase the degree of turbulence produced by the flat vane, a delrin peg is positioned, on the downstream side of the pivots, and which allows the vane to open to an angle which is 10° smaller than that produced by an identically shaped delrin vane, Figure (5.8).
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Graph</th>
<th>Hydrodynamic Delrin Vane</th>
<th>Time after Exsanguination Graph</th>
<th>Flat Delrin Vane</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound 1</td>
<td>40%</td>
<td>1b</td>
<td>Circuit: 18.2+0.265 t, 0.009</td>
<td>1 hr 4.5 hr 1 hr</td>
<td>a + b x t, s_b</td>
<td>7.6+0.295 t, 0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1b'</td>
<td>Time-jar: 19.5+0.099 t, 0.009</td>
<td>0.166</td>
<td>0.198</td>
<td>-19%</td>
</tr>
<tr>
<td>Greyhound 2</td>
<td>50%</td>
<td>2a</td>
<td>Circuit: -0.6+0.356 t, 0.007</td>
<td>1 hr 4.5 hr 2b</td>
<td>a + b x t, s_b</td>
<td>3.4+0.434 t, 0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2a'</td>
<td>Time-jar: 0.5+0.025 t, 0.003</td>
<td>0.357</td>
<td>0.397</td>
<td>-8%</td>
</tr>
<tr>
<td>Greyhound 3</td>
<td>45%</td>
<td>3b</td>
<td>Circuit: 14.0+0.346 t, 0.003</td>
<td>1 hr 4.5 hr 3a</td>
<td>a + b x t, s_b</td>
<td>4.5+0.368 t, 0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b'</td>
<td>Time-jar: 15.3+0.048 t, 0.006</td>
<td>0.322</td>
<td>0.356</td>
<td>-8%</td>
</tr>
<tr>
<td>Greyhound 4</td>
<td>40%</td>
<td>4a</td>
<td>Circuit: 0.9+0.329 t, 0.018</td>
<td>1 hr 4.5 hr 4b</td>
<td>a + b x t, s_b</td>
<td>8.5+0.394 t, 0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4a'</td>
<td>Time-jar: 1.5+0.079 t, 0.009</td>
<td>0.319</td>
<td>0.342</td>
<td>-28%</td>
</tr>
<tr>
<td>Greyhound 5</td>
<td>40%</td>
<td>5a</td>
<td>Circuit: 2.8+0.445 t, 0.017</td>
<td>1 hr 4.5 hr 5b</td>
<td>a + b x t, s_b</td>
<td>13.7+0.383 t, 0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5a'</td>
<td>Time-jar: 4.6+0.105 t, 0.010</td>
<td>0.210</td>
<td>0.231</td>
<td>+38%</td>
</tr>
<tr>
<td>Greyhound 6</td>
<td>50%</td>
<td>6b</td>
<td>Circuit: 7.2+0.270 t, 0.002</td>
<td>1 hr 4.5 hr 6a</td>
<td>a + b x t, s_b</td>
<td>4.0+0.374 t, 0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6b'</td>
<td>Time-jar: 7.4+0.057 t, 0.003</td>
<td>0.346</td>
<td>0.373</td>
<td>-62%</td>
</tr>
</tbody>
</table>

a= calculated initial hemolysis, mg%
b= calculated hemolysis rate, mg% / minute
t= pumping time, minutes
s_b= standard deviation in b, mg% / minute

** These refer to graphs shown in Figures (5.10), (5.11) and (5.12).

Table (5.1) Hemolytic comparisons between the hydrodynamic delrin vane and the flat delrin vane.
Figure (5.10): Hemolytic comparisons between the hydrodynamic delrin vane (80), (1b,2a), and the flat delrin vane (60), (1a,2b).
(see Table 5.1)
Hemolytic comparisons between the hydrodynamic delrin (80), (3b,4a), and the flat delrin vane (60), (3a,4b).
(see Table 5.1)
Figure (5.12): Hemolytic comparisons between the hydrodynamic delrin vane (A0), (5a,6b), and the flat delrin vane (60),(5b,6a).
(see Table 5.1)
The flat carbon vane, of reduced angle of opening, is hemolytically compared to the hydrodynamically shaped carbon vane. Except for the pumping duration, which has been limited to 2 hours. The experimental conditions are identical to those prevailing in the previous experiment.

The results of the hemolytic comparisons are given in Figures (5.13) and (5.14) and Table (5.2). These results clearly indicate that the marked increase in turbulence in the vicinity of the flat vane renders the valve much more hemolytic than the hydrodynamic carbon valve. In comparing these results with the previously described experiments with delrin vanes, it is clear that the hemolytic differences between the two flat vane designs increase markedly with small reductions in the angle to which the flat vane opens during systole. This seems to hold, despite the findings, reported below, indicating that pyrolytic carbon is less traumatic to the RBC than delrin.

Again, the above results would be qualitatively unaffected if the hemolysis rates in the time-jars are excluded.

III. Delrin and Pyrolytic Carbon Hydrodynamically Shaped Vanes

These identically shaped vanes, are hemolytically compared under the following conditions:

- Pump Chamber: Mk I
- Pumped Volume: 900 ml
- Flow Rate: 3.3 litres/minute
- Stroke Volume: 50 ml
- Frequency: 66 r.p.m.
- Pumping Period: 3 hours
- Systolic/Diastolic Time Ratio: 1/3
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>**</th>
<th>Hydrodynamic Carbon Vane</th>
<th>Time after Exsanguination</th>
<th>**</th>
<th>Flat Carbon Vane</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound</td>
<td>45%</td>
<td>1b</td>
<td>Circuit : $1.7+0.353t$, $0.011$</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>1a</td>
<td>$-0.8+1.975t$, $0.087$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1b'</td>
<td>Time-jar: $2.7+0.052t$, $0.003$</td>
<td></td>
<td></td>
<td>1a'</td>
<td>$0.7+0.037t$, $0.006$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net rate: $0.301$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound</td>
<td>38%</td>
<td>2a</td>
<td>Circuit : $0.7+0.284t$, $0.002$</td>
<td>1 hr</td>
<td>4.5 hr</td>
<td>2b</td>
<td>$1.6+0.999t$, $0.013$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2a'</td>
<td>Time-jar: $0.6+0.044t$, $0.006$</td>
<td></td>
<td></td>
<td>2b'</td>
<td>$1.6+0.062t$, $0.003$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net rate: $0.240$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound</td>
<td>50%</td>
<td>3b</td>
<td>Circuit : $0.0+0.157t$, $0.014$</td>
<td>4.5</td>
<td>1 hr</td>
<td>3a</td>
<td>$1.9+1.904t$, $0.133$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b'</td>
<td>Time-jar: $1.1+0.079t$, $0.000$</td>
<td></td>
<td></td>
<td>3a'</td>
<td>$-0.1+0.039t$, $0.002$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net rate: $0.078$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound</td>
<td>42%</td>
<td>4a</td>
<td>Circuit : $-1.1+0.247t$, $0.016$</td>
<td>1 hr</td>
<td>4.5 hr</td>
<td>4b</td>
<td>$8.0+0.997t$, $0.095$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4a'</td>
<td>Time-jar: $0.0+0.034t$, $0.004$</td>
<td></td>
<td></td>
<td>4b'</td>
<td>$0.0+0.077t$, $0.005$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Net rate: $0.213$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a = calculated initial hemolysis, mg%

b = calculated hemolysis rate, mg% / minute

s_b = standard deviation in b, mg% / minute

t = pumping time, minutes

** These refer to graphs shown in Figures (5.13) and (5.14).

Table (5.2) Hemolytic comparisons between the hydrodynamic carbon vane and the flat carbon vane of reduced angle of opening.
Figure (5.13): Hemolytic comparisons between the hydrodynamic carbon vane (80), (1b,2a), and the flat carbon vane of reduced angle of opening (50),(1a,2b). (see Table 5.2)
Hemolytic comparisons between the hydrodynamic carbon vane (80), (3b,4a), and the flat carbon vane of reduced angle of opening (50), (3a,4b). (see Table 5.2)
The results of the hemolytic experiments are shown in Figures (5.15), (5.16) and (5.17) and in Table (5.3).

These results clearly indicate that the form of the delrin material used is appreciably more traumatic to the flowing RBCs than pyrolytic carbon. This finding is significant, particularly when considering the fact that the flow of blood in the vicinity of both vanes is observed to be mostly laminar, (Figure (5.9)), i.e. the flow conditions are not particularly severe.

Again, the above findings would remain qualitatively unchanged, if the hemolysis rates in the time-jars are excluded.

The pyrolytic carbon surfaces used in this and previous experiments have not been very highly polished—certainly not to the extent of the observed shiny surfaces of the corresponding material used in the commercially available prosthetic valves. Due to the lack of technical facilities, the used carbon vanes have only been polished by rubbing the surfaces with diamond dust, of an average particle size of 25\(\mu\text{m}\).

In all experiments, the delrin surfaces, however, have not been polished. It is therefore possible that the degree of surface roughness of the delrin vane is an important contributor to RBC damage. This factor is investigated below.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Graph</th>
<th>Hydrodynamic Vane</th>
<th>Esaquination</th>
<th>Graph</th>
<th>Hydrodynamic Vane</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound 1</td>
<td>46%</td>
<td>1b</td>
<td>$10.8 \pm 0.100 t, 0.003$</td>
<td>4.5 hr</td>
<td>1a</td>
<td>$5.3 \pm 0.371 t, 0.019$</td>
<td>$0.048 - 0.347 \times 100$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1b'</td>
<td>$10.4 \pm 0.052 t, 0.006$</td>
<td>Net rate: 4.5 hr</td>
<td>1a'</td>
<td>$3.7 \pm 0.024 t, 0.001$</td>
<td>$0.347$</td>
</tr>
<tr>
<td>Greyhound 2</td>
<td>30%</td>
<td>2a</td>
<td>$0.7 \pm 0.048 t, 0.007$</td>
<td>1 hr</td>
<td>2b</td>
<td>$2.3 \pm 0.105 t, 0.005$</td>
<td>$-623%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2a'</td>
<td>$0.7 \pm 0.004 t, 0.000$</td>
<td>Net rate: 4.5 hr</td>
<td>2b'</td>
<td>$1.5 \pm 0.014 t, 0.001$</td>
<td>$-107%$</td>
</tr>
<tr>
<td>Greyhound 3</td>
<td>50%</td>
<td>3b</td>
<td>$5.9 \pm 0.191 t, 0.003$</td>
<td>4.5 hr</td>
<td>3a</td>
<td>$0.4 \pm 0.505 t, 0.007$</td>
<td>$-185%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3b'</td>
<td>$6.2 \pm 0.021 t, 0.001$</td>
<td>Net rate: 1 hr</td>
<td>3a'</td>
<td>$1.0 \pm 0.020 t, 0.000$</td>
<td>$-162%$</td>
</tr>
<tr>
<td>Greyhound 4</td>
<td>50%</td>
<td>4a</td>
<td>$0.3 \pm 0.157 t, 0.005$</td>
<td>1 hr</td>
<td>4b</td>
<td>$7.5 \pm 0.383 t, 0.012$</td>
<td>$-317%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4a'</td>
<td>$0.7 \pm 0.019 t, 0.003$</td>
<td>Net rate: 4.5 hr</td>
<td>4b'</td>
<td>$7.5 \pm 0.022 t, 0.003$</td>
<td>$-214%$</td>
</tr>
<tr>
<td>Greyhound 5</td>
<td>50%</td>
<td>5b</td>
<td>$6.5 \pm 0.130 t, 0.006$</td>
<td>4.5 hr</td>
<td>5a</td>
<td>$4.5 \pm 0.510 t, 0.011$</td>
<td>$-214%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5b'</td>
<td>$7.5 \pm 0.009 t, 0.001$</td>
<td>Net rate: 1 hr</td>
<td>5a'</td>
<td>$6.2 \pm 0.006 t, 0.000$</td>
<td>$-214%$</td>
</tr>
<tr>
<td>Greyhound 6</td>
<td>48%</td>
<td>6a</td>
<td>$0.7 \pm 0.127 t, 0.005$</td>
<td>1 hr</td>
<td>6b</td>
<td>$3.4 \pm 0.381 t, 0.010$</td>
<td>$-214%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6a'</td>
<td>$1.4 \pm 0.016 t, 0.004$</td>
<td>Net rate: 4.5 hr</td>
<td>6b'</td>
<td>$4.1 \pm 0.033 t, 0.007$</td>
<td>$-214%$</td>
</tr>
</tbody>
</table>

$a$ = calculated initial hemolysis, mg%
$t$ = pumping time, minute
$s_b$ = standard deviation in b, mg% / minute

** These refer to graphs shown in Figures (5.15), (5.16) and (5.17).

Table (5.3) Hemolytic comparison between identically shaped hydrodynamic vanes made from pyrolytic carbon and delrin.
Figure (5.15): Hemolytic comparisons between identically shaped hydrodynamic vanes made from pyrolytic carbon (1b, 2a) and delrin (1a, 2b). (see Table 5.3)
Figure (5.16): Hemolytic comparisons between identically shaped hydrodynamic vanes made from pyrolytic carbon (3b,4a) and delrin (3a,4b). (see Table 5.3)
Figure (5.17): Hemolytic comparisons between identically shaped hydrodynamic vanes made from pyrolytic carbon (5b,6a) and delrin (5a,6b). (see Table 5.3)
IV. Polished and Unpolished Hydrodynamically Shaped Delrin Vanes

Pyrolytic carbon surfaces have been polished by rubbing them with diamond dust ($\sim 25 \mu m$). As delrin is a relatively softer material, this polishing process could not be applied without distorting the configuration of the delrin vane. The delrin surfaces have therefore been polished with fine stainless steel wool.

A talysurf machine has been used to give a measure of the roughness of the polished and the unpolished surfaces. Traces from this machine are shown in Figure (5.18). The slope of the surfaces was due to the curvature of the two vanes, both of which were of hydrodynamic design. The figure clearly shows significant differences in the roughness of the two surfaces.

The two hydrodynamic delrin vanes have been hemolytically compared under conditions of flow rates etc., identical to those of the previous comparison, III. The hemolytic results are shown in Figures (5.19) and (5.20) and in Table (5.4). These results indicate insignificant hemolytic differences between the two surfaces. The differences shown in Table (5.4) are those that are attributed to the ageing of the RBCs of the second pumping runs.

Figure (5.18)
Traces taken by a talysurf machine showing the contours of the polished and the unpolished delrin surfaces.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Polished Hydrodynamic Delrin vane</th>
<th>Time after Exsanguination</th>
<th>Graph</th>
<th>Unpolished Hydrodynamic Delrin vane</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound 1</td>
<td>41%</td>
<td>1b Circuit: 0.7+0.648 t, 0.046 4.5 hr</td>
<td>1 hr</td>
<td>1a</td>
<td>-3.4+0.464 t, 0.019</td>
<td>0.628-0.449 x 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1b' Time-jar: 3.2+0.020 t, 0.004 Net rate: 0.628</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound 2</td>
<td>42%</td>
<td>2a Circuit: -1.6+0.601 t, 0.014 1 hr</td>
<td>4.5 hr</td>
<td>2b</td>
<td>-0.6+0.689 t, 0.013</td>
<td>+29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2a' Time-jar: 0.0+0.030 t, 0.004 Net rate: 0.571</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound 3</td>
<td>40%</td>
<td>3a Circuit: 1.3+0.525 t, 0.031 1 hr</td>
<td>4.5 hr</td>
<td>3b</td>
<td>-0.4+0.706 t, 0.019</td>
<td>-16%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3a' Time-jar: 0.3+0.016 t, 0.001 Net rate: 0.509</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greyhound 4</td>
<td>38%</td>
<td>4b Circuit: -2.7+0.650 t, 0.033 4.5 hr</td>
<td>1 hr</td>
<td>4a</td>
<td>0.1+0.434 t, 0.015</td>
<td>+32%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4b' Time-jar: 0.3+0.038 t, 0.001 Net rate: 0.612</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a= calculated initial hemolysis, mg%  
b= calculated hemolysis rate, mg% / minute  
s_b = standard deviation in b, mg% / minute  
t= pumpind time, minute  

** These refer to graphs shown in Figures (5.19 ) and (5.20 ).

Table (5.4) Hemolytic comparisons between polished and unpolished hydrodynamic delrin vanes.
Figure (5.9): Hemolytic comparisons between polished and unpolished hydrodynamically shaped delrin vanes. Polished vanes: 1b, 2a. Unpolished vanes: 1a, 2b. (see Table 5.4)
Figure (5.20): 
Hemolytic comparisons between polished and unpolished hydrodynamically shaped delrin vanes. Polished vanes: 3a, 4b. Unpolished vanes: 3b, 4a. (see Table 5.4)
V. T-Dracula and R-Dracula Blood Chambers

These two blood chambers have been hemolytically compared under the following conditions:

- Pumped Volume: 1200 ml
- Flow Rate: 3.3 litres/minute
- Stroke Volume: 50 ml
- Frequency: 66 r.p.m.
- Pumping period: 3 hours
- Systolic/Diastolic Time Ratio: 3
- Valves Used: Flat perspex vanes, fitted in cylindrical perspex housings.

Due to the relatively higher blood velocity, tangential to the walls of the chamber, the wall shear stress levels in the T-Dracula chamber are significantly higher than the corresponding stress levels in the R-Dracula chamber. By adopting a short diastolic time, the differences in the time-mean velocity of the blood entering the two chambers would thus be increased. Larger differences in the respective $\frac{1}{w}$ values for the chambers may thus be obtained. In both chambers, the ventricular-dead space has been maintained constant.

The results of the hemolytic comparisons, conducted for the two chambers, are shown in Figures (5.21), (5.22) and (5.23) and Table (5.5). The results clearly show the effects of the RBC ageing factor on the hemolysis rates of the various pumped blood volumes, i.e., the first pumping run produces less hemolysis than the second run irrespective of the blood chamber used. This ageing factor seems to 'drown' any hemolytic differences that may exist between the two chambers. A close examination of the "% change in rate" values, may faintly suggest that the radial chamber is slightly less traumatic to the flowing blood than the tangential chamber. However, the RBC ageing factor and the fact that the RBC membrane fragility varies within animals of the same species, indicate that the adopted procedure is not 'sensitive' enough to expose the small hemolytic differences, if any, between the two chambers.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Graph</th>
<th>T-Dracula Chamber</th>
<th>Time after Exsanguination</th>
<th>R-Dracula Chamber</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep 1</td>
<td>25%</td>
<td>1b</td>
<td>Circuit: 3.9 + 0.207 t, 0.008</td>
<td>4.5 hr 1 hr</td>
<td>1a</td>
<td>0.4 + 0.164 t, 0.005</td>
</tr>
<tr>
<td>Sheep 2</td>
<td>18%</td>
<td>2a</td>
<td>Circuit: 0.0 + 0.114 t, 0.007</td>
<td>1 hr 4.5 hr</td>
<td>2b</td>
<td>0.3 + 0.171 t, 0.007</td>
</tr>
<tr>
<td>Greyhound 3</td>
<td>42%</td>
<td>3b</td>
<td>Circuit: 3.2 + 0.412 t, 0.013</td>
<td>4.5 hr 1 hr</td>
<td>3a</td>
<td>2.0 + 0.309 t, 0.010</td>
</tr>
<tr>
<td>Greyhound 4</td>
<td>40%</td>
<td>4a</td>
<td>Circuit: 1.1 + 0.247 t, 0.006</td>
<td>1 hr 4.5 hr</td>
<td>4b</td>
<td>2.5 + 0.335 t, 0.016</td>
</tr>
<tr>
<td>Greyhound 5</td>
<td>42%</td>
<td>5b</td>
<td>Circuit: 4.7 + 0.441 t, 0.011</td>
<td>4.5 hr 1 hr</td>
<td>5a</td>
<td>1.4 + 0.308 t, 0.007</td>
</tr>
<tr>
<td>Greyhound 6</td>
<td>40%</td>
<td>6a</td>
<td>Circuit: 1.7 + 0.248 t, 0.009</td>
<td>1 hr 4.5 hr</td>
<td>6b</td>
<td>3.9 + 0.420 t, 0.014</td>
</tr>
</tbody>
</table>

\(a\) = calculated initial hemolysis, mg%

\(t\) = pumping time, minute

\(b\) = calculated hemolysis rate, mg% / minute

\(s_b\) = standard deviation in \(b\), mg% / minute.

** These refer to graphs shown in Figures (5.21), (5.22) and (5.23).

Table (5.5) Hemolytic comparisons between the Tangential-Dracual and the Radial-Dracula blood chambers.
**Figure (5.2):** Hemolytic comparisons between the T-Dracula chamber (1b,2a) and the R-Dracula chamber (1a,2b), (see Table 5.5).
Figure (5.22): Hemolytic comparisons between the T-Dracula chamber (3b, 4a) and the R-Dracula chamber (3a, 4b), (see Table 5.5).
Figure 5.23: Hemolytic comparisons between the T-Dracula chamber (5b,6a) and the R-Dracula chamber (5a,6b), (see Table 5.5).
VI. Mk I and T-Dracula Blood Chambers

These chambers have been hemolytically compared under the following conditions:

- **Pumped Volume**: 1200 ml
- **Flow rate**: 3.3 litres/minute
- **Stroke Volume**: 50 ml
- **Frequency**: 66 r.p.m.
- **Pumping Period**: 3 hours
- **Systolic/Diastolic Time Ratio**: 1/3
- **Valves employed**: Flat persex vanes fitted in cylindrical perspex housing.

In the Mk I pump chamber, the ventricular dead-space has been reduced to almost zero. The flexible diaphragm has been allowed to just touch the walls of the pump chamber. The ventricular dead-space in the T-Dracula chamber, however, is large (~200 ml).

The results of the hemolytic comparisons of these two chambers are shown in Figures (5.24) and (5.25) and in Table (5.6). As in the previous comparison, the RBC ageing factor seems to be the dominant contributor to the observed differences in the hemolysis rates of the two blood chambers. The hemolytic differences, if any, between the above two chambers, seem to be very small and insignificant. This seems to indicate that the stasis of blood in the T-Dracula chamber is not significantly hemolytic.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Graph</th>
<th>Mk I Pump Chamber</th>
<th>Time after Exsanguination</th>
<th>T-Dracula Chamber</th>
<th>% Change in hemolysis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(a + b \times t, s_b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep 1</td>
<td>23%</td>
<td>1b</td>
<td>Circuit: (3.5 + 0.205 t, 0.009)</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>1a</td>
</tr>
<tr>
<td>Sheep 2</td>
<td>25%</td>
<td>2a</td>
<td>Circuit: (0.6 + 0.169 t, 0.007)</td>
<td>1 hr</td>
<td>4.5 hr</td>
<td>2b</td>
</tr>
<tr>
<td>Greyhound 3</td>
<td>42%</td>
<td>3b</td>
<td>Circuit: (5.2 + 0.366 t, 0.023)</td>
<td>4.5 hr</td>
<td>1 hr</td>
<td>3a</td>
</tr>
<tr>
<td>Greyhound 4</td>
<td>45%</td>
<td>4a</td>
<td>Circuit: (3.1 + 0.289 t, 0.010)</td>
<td>1 hr</td>
<td>4.5 hr</td>
<td>4b</td>
</tr>
</tbody>
</table>

\(a = \) calculated initial hemolysis, mg\%
\(b = \) calculated hemolysis rate, mg\%/minute
\(t = \) pumping time, minutes,
\(s_b = \) standard deviation in \(b\), mg\%/minute.

** These refer to graphs shown in Figures (5.24) and (5.25).

Table (5.6) Hemolytic comparisons between the Mk I and the T-Dracula chambers.
Figure (5.24): Hemolytic comparison between the Mk I chamber (1b,2a) and the T-Dracula chamber (1a,2b). (see Table 5.6)
Hemolytic comparison between the Mk I chamber (3b,4a) and the T-Dracula chamber (3a,4b). (see Table 5.6)
VII. The Prosthetic Form of the Edinburgh Valve

An evaluation of the hemolytic characteristics of the prosthetic form of the Edinburgh valve has been attempted. This has necessitated the construction of a suitable pump chamber, in which the prosthetic valve could be incorporated.

The chamber, shown in Figure (5.26), has been machined out of perspex, with the valve orifice seating designed to accommodate various types of prosthetic valves, e.g. ball-and-cage, Bjork-Shiley, etc. The aortic side of the chamber has been specifically machined to simulate the configuration of the physiological aortic sinuses, (after Wieting (66)).

The above described chamber, has been loaded with the circuit used in the previous hemolytic comparisons. When the chamber is fitted with the prosthetic form of the Edinburgh valve, (shown in Figure (5.6)), the performance of the hydrodynamically shaped vane has been found to be unsatisfactory: specifically either the vane only opens to an angle of $\sim 35^\circ$ to a line perpendicular to the axis of the flow, or opens fully but with the production of severe regurgitation. This is in contrast to the vane behaviour in the cylindrical form of the valve. Changes in the hydrodynamic design of the vane, as well as changes in the parameters of the driving actuator and the circuit, have been found to have little effect on the performance of the vane: in many cases, the vane has been observed to flutter, 'double shut', or remain open during the entire flow cycle.

Using the same flow chamber, the performance of a ball-and-cage prosthetic valve has also been investigated. During

*The chamber was kindly provided by D. Taylor, a research colleague of this department.*
Figure 5.26: The blood pump chamber used for testing the performance of the prosthetic form of the Edinburgh Valve.
systole, the ball has been observed to open and shut regularly and thus sustain a forward flow, with relatively little regurgitation. However, the motion of the ball during systole, has been observed to be very erratic: the ball would spin wildly and oscillate irregularly in the cage.

The above findings are unaffected by the elimination of the vortex-like flow of liquid in the blood chamber, achieved by introducing a perforated circular disc or baffle in the pump chamber.

The above described behaviour of the two types of valves may be attributed to the inadequacy of the dynamic properties of the mock circulation, and the probable poor matching between the valve and the geometry of its immediate vicinity. These factors and the effect of the design of the vane-supporting ring of the Edinburgh valve on the performance of the vane are currently being investigated in this department.

In view of the above, plans for investigating the hemolytic characteristics of the prosthetic form of the Edinburgh valve, particularly of the geometry of its ring, had to be abandoned. However, two exploratory hemolytic experiments have been conducted, in which two behaviours of the vane have been compared: (a) during systole, the vane only opens to an angle of $35^\circ$ to a line perpendicular to the axis of flow, thus producing a considerable degree of turbulence in the vicinity of the vane, (b) the hydrodynamic vane, though opening to a corresponding angle of $80^\circ$, is nevertheless appreciably regurgitant.
These two valve behaviours have been hemolytically compared, under the following conditions:

- **Pumped Volume**: 1700 ml
- **Flow Rate**: 3.3 litres/minute
- **Frequency**: 66 r.p.m.
- **Stroke Volume**: 50 ml
- **Pumping Period**: 3 hours
- **Systolic/Diastolic Time Ratio**: 1/3
- **Inlet Valve**: Cylindrical form fitted with pyrolytic carbon vane, hydrodynamically shaped.

The results are shown in Table (5.7). These results indicate that the regurgitant valve is significantly more hemolytic than the turbulent valve. This finding is particularly surprising, in view of the fact that the observed flow across the regurgitant vane is mostly laminar.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Hematocrit</th>
<th>Regurgitant Hydrodynamic Carbon Vane</th>
<th>Time after Exsanguination</th>
<th>Turbulent Hydrodynamic Carbon Vane</th>
<th>% Change in Hemolysis Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greyhound 1</td>
<td>21%</td>
<td>Circuit: 0.5 + 0.309 t, 0.008 1 hr 4.5 hr</td>
<td></td>
<td>1.6 + 0.124 t, 0.005</td>
<td>0.309 - 0.124 x 100 = +60%</td>
</tr>
<tr>
<td>Greyhound 2</td>
<td>18%</td>
<td>Circuit: 0.9 + 0.289 t, 0.010 4.5 hr 1 hr</td>
<td></td>
<td>0.2 + 0.072 t, 0.006</td>
<td>+75%</td>
</tr>
</tbody>
</table>

\( a = \) calculated initial hemolysis, mg/
\( b = \) calculated hemolysis rate, mg%/minute
\( t = \) pumping time, minutes
\( s_b = \) standard deviation in \( b \), mg%/minute.

Table (5.7) Hemolytic comparisons between a regurgitant hydrodynamic carbon vane and an identically shaped carbon vane of reduced angle of opening (turbulent vane).
CHAPTER SIX

DISCUSSION AND CONCLUSIONS

In this work, the study of the traumatic characteristics of the pulsatile pump has been confined to a series of hemolytic comparisons between the various designs and construction materials of the blood chamber and its valves. No attempt has been made to compare this pump to other types of commonly used blood pumps. Such a comparison, preferably conducted in-vitro, would have to take into account the materials of construction and the conditions of blood flow within the respective circulations: e.g. if a roller pump were loaded with the mock circuit used in this work, the resultant steady flow might alter the hemolytic contributions of the mock loop.

Like the ventricle, the pump used in this study is of the positive displacement type. The motion of the diaphragm during systole is predetermined by the chosen parameters of the driving actuator. The dynamic flow characteristics of the pump are very similar to those of the human ventricle. This is shown by a comparison of the flow spectra of the pump output, Figure (3.11), and the corresponding physiological spectra for man, Figure (3.3). The resultant pressure spectra, however, show higher pressures at all frequencies than the corresponding human spectra. This is attributable to the relatively high input impedance of the adopted mock arterial tree.

In designing a mock arterial loop, the original intention was to utilise the circuit for both hemolytic and dynamic studies of the Edinburgh valve. The study of the dynamic characteristics
of this valve necessitates the faithful reproduction of the magnitude and relationship of the aortic pressure-flow waves, i.e. the replication of the input impedance of the systemic circulation. The attainment of this physiological characteristic in a hydraulic model unavoidably involves a very large surface area exposed to blood, a high surface/volume ratio and relatively high levels of wall shear stress – factors believed to contribute most to blood trauma. Dynamic studies of the valve had therefore to be abandoned. Consequently, the adopted model of the circulation, though successful in producing adequate valve behaviour, fails to reproduce the dynamic properties of the systemic arterial tree; the input impedance of the model is appreciably higher than the corresponding systemic impedance, and the model fails to reproduce the systemic phase relationship between the pressure and flow waves. Furthermore, the system of flow in the mock circuit is linear only for the first few harmonics, (Figure (3.9)); i.e. the behaviour of the circuit is frequency dependent.

The above described dynamic properties of the mock arterial tree may explain the unsatisfactory performance of the prosthetic form of the Edinburgh valve when inserted in the outlet site of the pump. Another factor that may affect the performance of this form of the valve is the probable poor matching between the design of the vane-supporting ring and the geometry of the pump chamber in its immediate vicinity. It is likely that this feature is common to other types of prosthetic valves. It also seems possible that in view of the above, the performance of prosthetic valves in in-vitro circuits of inadequate dynamic characteristics may not be reproduced in a corresponding site in-vivo.
The surface/volume ratio of the adopted mock cardiovascular loop is $1.2 \text{ cm}^{-1}$. This compares most favourably with other discussed models of the circulation: $27 \text{ cm}^{-1}$ for the Hydrospace model and $89 \text{ cm}^{-1}$ for the Westerhof model. Furthermore, the conduits of the adopted mock arterial tree are smooth and relatively large ($\geq 1.3 \text{ cm I.D.}$). Although it is difficult to evaluate in isolation the hemolytic properties of the mock tree, the above mentioned features will most likely appreciably reduce its traumatic contributions. The fact that hemolytic differences were observed between some of the studied factors indicated that these differences were not 'drowned' by the hemolytic contributions of the mock tree. (This is in contrast to the magnitude of the hemolytic contributions of the RBC ageing factor - see below.)

Outdated human blood has frequently been used in the viscometric investigations of the mechanical properties of the RBC membrane. As this blood contains a significant proportion ($\sim 20 - 30\%$) of hemolysed and severely damaged cells, results obtained with outdated blood should be evaluated with care. In this study, results obtained with pooled batches of this blood were not reproducible. It is likely that the pooling of blood from various individuals, though of the same grouping and factor, may have contributed to the observed levels of hemolysis. With fresh ox blood, on the other hand, very little hemolysis was observed even after long periods of pumping ($\sim 7$ hours). This has been attributed to the 'toughness' of the RBC membrane and its capacity to absorb a considerable degree of sub-lethal damage without rupture.

In view of the above, fresh blood was obtained by the exsanguination of greyhounds and sheep. As the amounts obtained
from single animals were not sufficient for the intended hemolytic studies, the blood was diluted with various amounts of Ringer-Lactate solution. The dilution of blood with this solution was found to have no immediate or delayed effects on the observed hemolysis rates of the pumped blood. However, the relationship between the hematocrit levels of greyhound blood, which depended on the degree of dilution, and the hemolysis rates of the pumped blood was found to vary disproportionately; e.g. by increasing the hematocrit level by a factor of 3, an increase in the hemolysis rate by a factor of 8 was observed (all else maintained constant). This suggests that when comparing two experiments conducted with greyhound blood, but of varying hematocrit levels, the hemolytic results of the blood of the lower hematocrit are very likely to be disproportionately reduced.

RBCs of donated human and animal blood undergo various changes, of a biochemical nature, which with time render their membranes very fragile. As a result RBCs of donated human blood are used for re-infusion within 21 days of the donation date. The time-jar experiments which have been conducted on exsanguinated animal blood clearly indicate that the fragility of the RBC membrane increases measurably within the first few hours of the exsanguination process. This effect is referred to here as the RBC ageing factor.

Due to the availability of only one actuator mechanism, hemolytic comparisons between any two factors were necessarily conducted consecutively. The time lapse between the end of the exsanguination process and the start of the first pumping run was \(~1\) hour. The corresponding time lapse for the second pumping run was \(~4.5\) hours. These time lapse differences have been found to
affect the hemolysis rate significantly for all blood pumping runs. Specifically, as in the case of the time-jar experiments, the hemolysis rate in the second of two pumping runs has been found to be always higher than the hemolysis rate of the first run (all else maintained constant). Alternating the factors under study in consecutive experiments was therefore essential.

The above findings necessarily imposed some limitations on the sensitivity of the adopted procedure in detecting hemolytically significant design and material changes. Thus only those changes whose traumatic effects were large enough to overcome the additional hemolysis due to the ageing factor were exposed. In the series of 9 hemolytic comparisons, described in Chapters Four and Five, 4 comparisons, viz. Comparisons IV, V, VI and that given in Table (4.5), were found to produce no detectable hemolytic differences. In these, the hemolysis rate of the second of the two pumping runs of each experiment was always found to be higher than the corresponding rate of the of the first run.

The hemolysis rates produced by the time-jars were used in an attempt to compensate for the RBC ageing factor; these rates were subtracted from the corresponding hemolysis rates produced by each of the studied factors. However, as the tabulated results of the previous chapter show, this method of approximation proved to be inadequate; the time-jar rates were relatively very low and all results remained qualitatively unchanged when the time-jar rates were excluded.

The results of the hemolytic comparisons between the various adopted designs and materials of vanes of the Edinburgh valve are listed in Table (6.1). In this table the asterisks denote
the less hemolytic of each of the two compared factors. The percentage figures shown are the arithmetic mean of the "% change in hemolysis rate" included in the tabulated results of the previous chapters. The "% changes" have been calculated thus:

\[
\frac{\text{hemolysis rate for less traumatic factor}}{\text{hemolysis rate for more traumatic factor}} \times 100
\]

As blood of different greyhounds is used for each experiment and the fact that the RBC membrane fragility can vary from one greyhound to the next, the arithmetic mean cannot be regarded as an accurate overall measure of the observed hemolytic differences. It is used here however, to provide a rough quantitative basis for comparing the results of the various pairs of studied factors.

Comparison

I  ** hydrodynamic delrin vane (80)  # flat delrin vane (60)  -25%

II  ** hydrodynamic carbon vane (80)  flat carbon vane (50)  -860%

III  ** hydrodynamic carbon vane (80)  hydrodynamic delrin vane (80)  -270%

IV  polished hydrodynamic delrin vane (80)  unpolished hydrodynamic delrin vane (80)  0%

** the less hemolytic of the two compared factors.
# this refers to the angle to which the vane opens during systole; i.e. the angle between a line perpendicular to the axis of flow and the vane axis, (Figure (5.8)).
As Table (6.1) shows, the adopted changes in the designs and the materials of construction of the vanes of the valve have proved to be hemolytically significant; e.g. (a) the flat delrin vane (60°) produces a higher rate of hemolysis than the hydrodynamic delrin vane (80°), and (b) the hydrodynamic carbon vane (80°) produces a lower hemolysis rate than the identically shaped delrin vane (80°). Furthermore, the table suggests that qualitatively, the order of the examined vanes, in terms of increasing traumatic properties are:

- hemolysis increasing
- hydrodynamic vane
- delrin vane
- flat delrin vane
- flat carbon vane

Comparisons I and III indicate that the adopted change of materials has a greater traumatic effect than the adopted change in the vane design. This suggested that, compared to pyrolytic carbon, the form of delrin used is hemolytically unsuitable, and that the mere exposure of blood to this material may cause appreciable degree of hemolysis. To investigate this, two identical vanes, one delrin and the other pyrolytic carbon were each placed in a separate time-jar, each containing 100 ml of blood of the same greyhound. The jars were simultaneously rotated, at 66 r.p.m. for a period of 3 hours. The differences in the hemolysis rates in the two jars were observed to be insignificant. (The same result was obtained with two identical vanes, one made of perspex and the other of pyrolytic carbon.) It is therefore inferred that hemolytic differences between delrin and pyrolytic carbon only manifest themselves when the hydrodynamic conditions are sufficiently severe.

It also seemed possible that the roughness of the
delrin surfaces might have significantly contributed to the observed unfavourable hemolytic characteristics of the material. Comparison IV however, shows that variations in the roughness of the delrin surface, of the order of 2 to 3 μm, are hemolytically insignificant. This seems to indicate that other more intrinsic properties of the delrin surface, possibly its surface chemistry and electrical charge, are largely responsible for its traumatic characteristics.

Comparison I and III also suggest that the changes in the flow conditions resulting from the adopted design changes of the vanes may not have been appreciable. In Comparison II, the angle to which the flat carbon vane opens during systole has been reduced by 10. Comparisons I and II clearly indicate that the resultant increase in the turbulence created in the vicinity of the flat carbon vane (50°), results in appreciably higher hemolytic differences than those obtained for the less turbulent flat delrin vane (60°) - this seems to hold inspite of the fact that the carbon material is appreciably less traumatic than delrin. This is supported further by an examination of the magnitudes of the hemolysis rates produced by each of the flat vanes, (Tables (5.1) and (5.2)); the maximum hemolysis rate produced by the flat delrin vane (60°) is 0.434 mg%/minute and the minimum hemolysis rate produced by the flat carbon vane (50°) is 0.992 mg%/minute. Such differences are too large to be solely attributed to the variations in the fragilities of RBCs of various greyhounds.

The adopted changes in the design of the blood pump chamber were found to be hemolytically insignificant. Specifically, the relative increase in the wall shear stress level within the
T-Dracula chamber and the non-uniform blood flow pattern in the
R-Dracula chamber, were found to be equally traumatic. Furthermore,
the appreciable increase of the end-systolic volume and the con­
sequent blood stasis developed in the T-Dracula chamber, as compared
to those within the Mk I chamber, were found to be hemolytically
insignificant. It seems likely that the differences in the blood
flow patterns, in terms of developed fluid and wall shear stress
levels, resulting from the above design changes in the pump chamber,
are not as appreciable as those produced by the design changes
in the valves.

The results of the experiments reported in this
work suggest that valve design plays a major role in dictating
the overall traumatic characteristics of a pulsatile blood pump.
This crucial hemolytic role may be attributed to the blood flow
conditions in the vicinity of these valves. The fluid and the
wall shear stress levels in the vicinity of the inlet and outlet
valves, particularly the latter, are most likely to be higher
than those prevailing anywhere within the pump chamber or at its
blood exposed surfaces. This is amply manifested by the appreciable
traumatic effects that result from changes in the material of
construction of the occluders of the inlet and outlet valve. The
combined surface areas of these occluders are but a very small
fraction of the total pump area exposed to the flowing blood. Yet,
because the flow conditions in the vicinity of these occluders
are particularly severe, the rate of cell-surface collisions at
the occluder surfaces is likely to be higher than the corresponding
rate for the pump surfaces. It is probable that for a given valve
design, the hemolytic differences between various materials of construction are amplified by the severity of conditions which RBCs are subjected to by the design factors; if the design factors contribute to the frequency and the severity with which RBCs collide with a given surface, then the outcome of these collisions will depend on both the nature of the surface and the severity of the RBC-surface collision process.

As discussed in Chapter 2, the hemolytic contributions of the fluid forces developed in the adopted loop may be attributed to their effects acting directly on the RBC (a) remote from non-biological surfaces and (b) in the vicinity of these surfaces.

Remote from non-biological surfaces, the maximum level of fluid shear stress $\tau$ is developed in the turbulent regions in the vicinity of the occlusive valves; as shown in Appendix D, the fluid flow $\tau$ in all other conduits of the loop is laminar. The fluid shear stress level in these turbulent regions cannot be deduced theoretically. However, in view of the relatively low average fluid velocity across the valve (43 cm/sec) and the low (average) RE Number of the flow (less than 2000), the maximum fluid stress level seems unlikely to exceed the order of $10^2$ dynes/cm$^2$. The reviewed literature indicates that at fluid shear stress levels below $10^3$ dynes/cm$^2$, outright cell rupture, remote from non-biological surfaces is unlikely. It is suggested that for the adopted valve designs, the hemolytic contribution of the turbulent fluid regions is most likely to be confined to some form of sub-lethal damage.

In the adopted loop the regions of maximum levels of wall shear stress, $\tau_w$, exist at (a) the blood exposed surfaces of the smallest conduit and (b) at surfaces in the turbulent vicinity of the occlusive valves. Estimates of these (Appendix D) show that the wall shear stress values for both regions are well below $10^2$ dynes/cm$^2$. For this level of $\tau_w$ and in the light of the reviewed literature, the following suggestions are put forward:

(i) RBCs are unlikely to rupture on impact with the surface.
(ii) If the level of $\tau_w$ is maintained above 10 dynes/cm$^2$ throughout the cycle, then the adhesion of the RBCs to surfaces and adhesion related damage, are unlikely. However, during the flow cycle,
e.g. when the vanes are momentarily closed, \( T_c \) may drop below 10 dynes/cm\(^2\) long enough to allow cells to adhere to the non-biological surfaces. Such adherent cells will most likely be detached during systole, thereby sustaining a form of sub-lethal damage (7).

(iii) A most likely source of cell damage seems to result from the RBCs colliding with and reflecting away from the non-biological surfaces. It seems that during the brief duration of the cell-surface contact the RBC membrane undergoes a certain degree of sub-hemolytic damage. Successive collisions with the surfaces of the loop ultimately leads to cell rupture.

Figure (6.1) has been suggested by Blackshear (17) to predict the hemolysis resulting from the flow of blood in tubes of varying diameters. The heavily marked area, Region A, denotes the region within which the flow conditions in the 1/2 inch tubing, shown in Figure (3.7), lie. According to the figure, cell damage in this region is most likely to occur as a result of cells sticking to surfaces which partly agrees with what has been suggested earlier. The figure does not indicate that damage (sub-lethal), resulting from cell-wall collisions can occur at low average fluid velocities (less than 500 cm/sec).

Blackshear's diagram is for tubes only and its use cannot be reliably extended to include the flow regions in the vicinity of occlusive valves. Furthermore, Figure (6.1) has the following limitations:

(i) It does not take into account the viscoelastic nature of the RBC membrane; the time duration of the developed fluid forces must be taken into account.

(ii) As mentioned on pages 6 and 10 of the reviewed literature, RBC damage does not seem to be related to the fluid shear rate; Blackshear's diagram seems to imply that it does.

(iii) The figure does not differentiate between outright cell rupture and sub-hemolytic damage. Furthermore, due to the presence of sub-lethal damage the outlined boundaries of the diagram must necessarily be 'diffuse'. It is suggested that these boundaries should be presented in the form of regions rather than clear cut lines.

Furthermore, it is the confluence of the fluid forces acting directly on the RBCs and the nature of the non-biological surfaces with
**Figure (6.1):** Domains of fully developed flow of blood in a tube: Blackshear (17). Region A, marked by a spot, indicates the prevailing conditions in the 1/2 inch tubing used in the adopted blood circuit (Figure 3-7).

**Figure (6.2):** Suggested domains of cell damage in blood perfusion circuits. Shaded area indicates the region within which the conditions in the adopted blood circuit (Fig. 3-7) prevail.
which RBCs collide that dictate the overall hemolytic characteristics of an in-vitro pumping circuit. This does not seem to be emphasised enough by Blackshear (17).

Figure (6.2) which takes account of the viscoelastic nature of the RBC membrane and the various levels of the fluid and wall shear stress is proposed. The figure is based on the experimental findings reported in the literature and which are indicated by their reference numbers. The figure also includes the regions within which the fluid and wall shear stress levels seem to prevail in the adopted circuit during any flow cycle.

In this work the study of hemolysis in a pulsatile pump has been confined to a series of hemolytic comparisons between various designs and construction materials of the blood chamber and its valves. In the light of the information yielded in the preliminary experiments described in Chapter 4, hemolytic comparisons between various types of blood pumps (pulsatile, roller etc.) must necessarily be carried out under strict and controlled conditions. These include:

(a) The blood used must be of the same animal and of the same hematocrit value.

(b) Due to the RBC ageing factor, hemolytic comparisons must be conducted simultaneously.

(c) The hemolytic contribution of the mock circulation must be identical for each of the compared pumps. This clearly depends on the flow conditions in each loop, e.g. for two identical mock arterial systems, one fitted with a roller pump and the other with a pulsatile pump, the flow in the former will be almost steady, whereas the flow in the latter will be pulsatile. The hemolytic contribution of the loop will also depend on its material of construction.

(d) The method of determining the free plasma haemoglobin must be the same for all comparisons; results obtained with different methods can give varying results, (see Appendix B and Ref. 67).

(e) The flow settings of any studied pump must be strictly defined, e.g. (i) for a pulsatile pump, the frequency and the stroke volume must be taken into account; it cannot be claimed that hemolysis in the pump will only be dependent on their product, namely the flow rate,
<table>
<thead>
<tr>
<th>Pump</th>
<th>Hemolysis**</th>
<th>Flow Rate L/min.</th>
<th>Blood</th>
<th>Hematocrit</th>
<th>Method of determining Free Plasma Haemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capalletti et al</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(67)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roller</td>
<td>0.37</td>
<td>2.2</td>
<td>fresh mongrel unspecified</td>
<td></td>
<td>Beckman (67)</td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0.9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>'Finger'</td>
<td>1.02</td>
<td>2.1</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.8</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Diaphragm (pulsatile)</td>
<td>0.9</td>
<td>1.9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>0.9</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Brown et al</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(68)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roller</td>
<td>0.004</td>
<td>2.0</td>
<td>used human#</td>
<td></td>
<td>Coleman (68)</td>
</tr>
<tr>
<td>Pulspirator</td>
<td>0.027</td>
<td>2.0</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Siedel-Curtis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bellofram (pulsatile)</td>
<td>0.020</td>
<td>2.0</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Alami (work described in this thesis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macleod Diaphragm (pulsatile)</td>
<td>0.107</td>
<td>3.3</td>
<td>fresh blood of various greyhounds</td>
<td>45%</td>
<td>Cyanamethamoglobin (see Appendix B)</td>
</tr>
<tr>
<td></td>
<td>0.030</td>
<td>3.3</td>
<td>&quot;</td>
<td>46%</td>
<td>&quot;</td>
</tr>
<tr>
<td>Roller##</td>
<td>0.19</td>
<td>4.8</td>
<td>&quot;</td>
<td>40%</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>0.069</td>
<td>4.8</td>
<td>&quot;</td>
<td>40%</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

* blood previously used in open heart surgery
** gram/litre of free g hgn per 100 litres pumped
# pump fitted with pyrolytic carbon Edinburgh valves
## the circuit is shown in fig. 3.7
### the circuit is shown in fig. 4.1

**TABLE 6.2**
(ii) the degree of occlusion of a roller pump must be specified.

In view of the above, comparisons of the hemolytic characteristics of various pumps obtained by different workers under varying experimental conditions cannot reliably provide useful information about the relative merits of each pump. The above conditions would still have to be met even when, as has been suggested#, comparisons are conducted via the use of indices of hemolysis. These indices may be based on a variety of working conditions, e.g.

(i) % hemolysis; defined as $\frac{\text{Free plasma haemoglobin released}}{\text{Total blood haemoglobin}}$  
(N.B. This index will increase with increasing fragility of the RBC).

(ii) Free plasma haemoglobin released per unit volume pumped or per number of passages of a unit volume round the loop.
(N.B. These indices may depend on the various settings of a given pump, see condition (e) listed above).

Table (6.2) shows hemolytic data obtained by three different groups of workers in which various pumps were compared under varying experimental conditions. The calculated indices of hemolysis clearly show how the use of different experimental conditions result in widely varying and incomparable results; the three groups of workers, who used three different circuits, have obtained different ranges of hemolysis indices. Even in the cases where similar experimental conditions have been employed (67), the results show the indices of hemolysis to be dependent on the flow rate. The table also shows how indices can vary when blood of different animals, though of the same species, is used in the assessment of the same pump, e.g. under identical conditions, the Macleod Diaphragm pump gives widely varying indices when blood of different greyhounds is used. Furthermore, the three teams of workers have used three different methods of free plasma haemoglobin determination.

Clearly, the above indicates, that contrary to the suggestion put forward by Bellhouse#, hemolytic results of various pumps, even when obtained in the form of indices, can only be compared when the experimental conditions are maintained the same for each of the compared pumps.

# Dr. B. Bellhouse, Mechanical Eng. Dept., Oxford University, U.K.
Appendix A

Fourier Series

A periodic signal, p(t), can be decomposed into a series of sinusoidal components, the so-called harmonics:

\[ p(t) = A_0 + A_1 \cos \omega t + A_2 \cos 2\omega t + \ldots + A_m \cos m\omega t + B_1 \sin \omega t + B_2 \sin 2\omega t + \ldots + B_m \sin m\omega t. \]

where \( A_i = M_i \cos \varphi_i \)
\( B_i = M_i \sin \varphi_i \)

and \( M_i = \sqrt{A_i^2 + B_i^2} \)
\( \varphi_i = \arctan \left( \frac{B_i}{A_i} \right) \quad i = 1, 2, 3, \ldots, (m-1) \)

\( M_i, \varphi_i \) = modulus and phase of \( i \)th harmonic,

\( A_0 \) = steady component,

\( m \) = number of highest harmonic of interest,

\( \omega = \frac{2\pi}{T} \)

\( T \) = duration of cycle.

If the \( (p) \) signal is digitised into \( \Delta t \) equal intervals, the above Fourier coefficients may be computed from the \( N \) samples available:

\[
A_0 = \frac{1}{N} \sum_{k=0}^{N-1} p(k \Delta t) \\
A_i = \frac{2}{N} \sum_{k=0}^{N-1} p(k \Delta t) \cos(2\pi ik/N) \\
B_i = \frac{2}{N} \sum_{k=0}^{N-1} p(k \Delta t) \sin(2\pi ik/N)
\]

where \( k = 0, 1, 2, \ldots, (N-1) \).

The above procedure must be applied to both the pressure and the flow signals.
Appendix B

Measurement of Free Plasma Haemoglobin Concentration

Two methods have been considered, viz. the Benzidene and the Cyanmethaemoglobin methods. As the former involves the handling of benzidene hydrochloride (a carcigenous compound), its use was ruled out for safety reasons.

The basis of the cyanmethaemoglobin method is to dilute whole blood in a solution containing potassium cyanide and potassium ferricyanide (Drabkin's Reagent). Haemoglobin, methaemoglobin and carboxyhaemoglobin are all rapidly converted to cyanmethaemoglobin. The absorbance of the solution is then measured in a photo-electric colorimeter at the appropriate wavelength (540 nm or 415 nm).

Drabkin's Reagent is commercially available in the form of pellets readily soluble in distilled water, e.g. Aculute Diluent Pellets (Ortho Pharmaceutical Ltd., U.K.).

As mentioned above this method applies to haemoglobin measurements for whole blood. However, it has been adapted to measure the free plasma haemoglobin concentrations of the samples withdrawn in the conducted hemolytic experiments.

The adaptation of the cyanmethaemoglobin method consisted mainly in using larger volumes of plasma to compensate for the small amounts of haemoglobin contained in the test samples:

1. A Drabkin solution, referred to here as the diluent, is prepared by dissolving one Aculute pellet in 250 ml of distilled water.

2. A 5 ml solution is prepared with the following composition:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>diluent</td>
</tr>
<tr>
<td>x ml</td>
<td>plasma test sample</td>
</tr>
<tr>
<td>4-x ml</td>
<td>distilled water</td>
</tr>
</tbody>
</table>

   The volume x, depends on the concentration of the haemoglobin in the test sample and can vary from 0.25 ml to 4 ml. The volume x, should be such that the measured Optical Density (O.D.) of the prepared 5 ml solution lies between 0.05 and 2.0 — within this range, the errors in reading the O.D. are minimal.

3. The prepared 5 ml solution is allowed to stand for 10 minutes.

*Saunderton, High Wycombe, Bucks., England.*
before reading its O.D. The obtained reading is then compared to the O.D. s of standard solutions of cyanmethaemoglobin. The blank solution against which all samples are read in the spectrophotometer, has the following composition:

1 ml diluent + 4 ml distilled water.

(4) The standard solutions of accurately known concentrations of cyanmethaemoglobin are commercially available, e.g. Acuglobin solution (Ortho Diagnostics, Division of Ortho Pharmaceutical Ltd.). A relationship between the O.D.² and the concentrations of these standards is obtained. This relationship is found to be nearly linear, e.g.

<table>
<thead>
<tr>
<th>Composition of standard samples</th>
<th>O.D. at 415 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard</td>
<td>d. water</td>
</tr>
<tr>
<td>3 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>0.5</td>
<td>3.5</td>
</tr>
<tr>
<td>0.25</td>
<td>3.75</td>
</tr>
</tbody>
</table>

The O.D. is plotted against the known concentrations and the straight line graph is used to obtain the unknown concentration of the test sample; the concentration of the test sample is directly read from the graph and multiplied by the appropriate dilution factor.

Notes:

(i) The absorbance of the prepared solutions was measured at a wavelength of 415 nm as opposed to 540 nm. For both wavelengths the 'O.D. - concentration' relationship is linear. However, due to the higher light absorption at the 415 nm wavelength, the slope of the line 'O.D. - concentration' is greater and the errors involved are therefore smaller.

(ii) The results obtained with this method are more consistent than those obtained with the benzidine method; e.g. the two methods were compared using two stocks of plasma, p1 and p2:

<table>
<thead>
<tr>
<th>Composition of p1 plasma samples</th>
<th>Calculated concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benzidine method</td>
</tr>
<tr>
<td>Sample</td>
<td>p1</td>
</tr>
<tr>
<td>a</td>
<td>3 ml</td>
</tr>
<tr>
<td>b</td>
<td>2</td>
</tr>
<tr>
<td>c</td>
<td>1.5</td>
</tr>
<tr>
<td>d</td>
<td>1</td>
</tr>
</tbody>
</table>
Calculated concentrations

<table>
<thead>
<tr>
<th>Composition of p2 plasma samples</th>
<th>Benzidine method</th>
<th>Cyanmeth. method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample</strong></td>
<td><strong>p2</strong></td>
<td><strong>d. water</strong></td>
</tr>
<tr>
<td>e</td>
<td>3ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>f</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>g</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>h</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Strictly, both methods were specifically designed for the measurement of haemoglobin in whole blood. The adopted method however, seems more consistent and reliable in measuring the large concentration of haemoglobin in plasma; e.g. doubling the added volume of the p1 stock (from 1 ml to 2 ml) almost doubles the derived concentration (from 5.2 mg% to 10.1 mg%). It is known that the results of the two methods cannot vary. For the conducted hemolytic experiments in this study, however, the consistency of the adopted method is more essential than the determination of the absolute concentration of haemoglobin in the free plasma.

(iii) The errors involved in the adopted method are small and consist of: (a) errors in preparing the 5 ml solution, e.g. errors inherent in use of pipettes, burette, etc. This is estimated at not more than 2%. (b) errors involved in preparing diluted standard solutions, estimated at not more than 2%. (c) Errors involved in deriving the 'O.D. - concentrations' relationship. This is estimated at not more than 1.5%. The total error is therefore estimated at not more than 5.5%.
Appendix C

By N. Macleod, Chemical Engineering Dept., Edinburgh University, Scotland, U.K.

By N. Macleod, Chemical Engineering Dept., Edinburgh University, Scotland, U.K.

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The Geometry of Smooth-Bore Non-Return Valves for Controlling Blood Flow:
A Study in Pseudo Three-Dimensional Mechanical Design.

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Introduction

All non-return valves consist essentially of a housing, forming part of the flow channel, and one or more movable components contained within it and capable of occluding it. Such occluders must be coupled to the housing in a manner permitting movement by fluid mechanical forces between two positions, one blocking the flow and the other affording relatively free passage.

Valves designed to control the pulsatile flow of blood - prosthetic inlet and outlet valves for the natural human heart, for instance, or for a pump serving as a heart substitute - are required to open rapidly when acted on by a small forward pressure gradient, and to offer the minimum of obstruction when flow is established. Now the occluder will move rapidly into the fully open position at the commencement of a pulse only if the hydrodynamic forces acting on it are then relatively large; but when the valve is fully open, any such large force in the streamwise direction will correspond to a large hydraulic resistance. The two requirements of rapid opening and low pressure drop cannot both be satisfied fully, therefore, if the occluder is coupled to the housing in a manner which restricts its motion to pure sliding in the direction of the stream. In principle, however, they can be completely satisfied in a valve in which the occluder rotates from the occlusive to the non-occlusive position about an axis perpendicular to the stream direction. If, for instance, the occluder is a thin vane or disc, it can be arranged to block the flow when set normal to the channel, and to offer very little resistance when rotated about a chord into a plane nearly parallel to the flow. The torque tending to rotate the vane from the occlusive position may nevertheless be large if the vane axis is offset from the centre of pressure.

Several such tilting-vane valves for controlling blood flow have been described in recent years, and at least one of these [1] has shown some promise in prosthetic applications in place of the poppet valves still used almost exclusively as human heart-valve replacements [2]. All tilting-vane valves so far described, however, have a characteristic which impairs their performance in general and which makes them quite unsuitable for use in 'artificial hearts' or blood-pumps. This undesirable feature is the presence of projecting rim-like shelves or abutments extending around the inner surface of the housing, providing annular surfaces normal to the flow direction against which the faces of the disc can seal.* Such discontinuities or protrusions at the walls of the blood channel necessarily promote local turbulence and increase the resistance to flow. But in addition, they are particularly objectionable when the

*The Björk valve (1) lacks this feature, but does not seat positively.
adjacent parts of the channel walls are of artificial material, as when the valve forms part of a mechanical heart substitute. In this case, the stagnant fluid in the wall region immediately downstream of the abutment is likely to yield thrombi, or clots, most hazardous for the organism in the pump circuit.

Smooth-Bore Pivotted-Vane Valves

At an early stage of the work of the Edinburgh Artificial Heart Group, these considerations led us to develop tilting-vane valves with smooth-bore housings, in which the walls of the flow passage are entirely free of discontinuities but capable of providing a liquid-tight seal against the edges of a pivotted occluder. Such valves are now in regular use in our pumps and their prosthetic application is under investigation. The essential geometrical principle of their construction is that in a smoothly tapered tube of suitable form a vane or plate, not necessarily plane, touching the walls around the entire perimeter in a zone of finite width, may be rotated about an axis offset with respect to its centre of fluid pressure into a plane more nearly parallel to the tubular axis. For any particular form of housing having this property the valve designer requires to determine the location of axes about which this rotation may take place. We here discuss the solution to this problem, no previous treatment of which is known to the author.

Preliminary Considerations

It can be assumed that the housing of any practical valve must be a body of revolution. It is easy to see, however, that a cylindrical housing will not permit the required rotation of an occluding plate about any offset axis, such as BB' in Fig. 1. The diameter AA' of the plate is longer than any of the chords of the cylinder with which it might be made coincident by rotation about BB'; part of the perimeter of the plate would therefore penetrate the cylindrical surface in such a rotation. The only transverse axis about which the plate can rotate is AA'; but this is an axis of symmetry, passing through the centre of pressure, about which there can be no turning moment due to a pressure difference across the faces of the plate.

It is therefore clear that the housing of any pivotted-plate non-return valve, capable of operation by internal differences of fluid pressure, must have a cross-section which varies along the principal axis. For such a bell- or trumpet-shaped body, all chords lying on the cylindrical surface generated by a finite rotation of AA' about BB' may be longer than AA', the paths of whose extremities may thus lie entirely within the body. For a suitably chosen axis of rotation, the coaxial paths traced by the extremities of all chords of the plate when the latter turns into a position parallel to the axis of the body may similarly lie wholly within the body.

The simplest body of revolution of variable section is the right
circular cone. We shall here restrict our discussion to this case, for which we will now determine the location of suitable axes for vane rotation. As a further simplification, we shall develop our analysis on the assumption that the vane is a flat plate, and we will consider only axes of rotation parallel to the plane of the vane and to the base of the cone. The treatment given is, however, readily generalised to cover all possible cases.

The Rotation of a Lamina within a Cone

Suppose that the lamina QPP'Q' occluding the right circular cone shown in Fig. 2 is cut in PP' by a plane perpendicular to the required axis or axes of rotation. This plane cuts the cone in RPP'R'. If the lamina is to rotate through some finite angle without penetrating the conical surface, the circular arcs traced by P and P' in the plane of section during that rotation must lie wholly within the curve RPP'R'; and the required rotation of the lamina can occur if, and only if, the corresponding conditions are simultaneously fulfilled in all parallel planes cutting QPP'Q'.

It follows that, although the geometry of the system is three dimensional, the existence and location of suitable axes of rotation can be determined from a plane diagram formed by projecting such curves as RPP'R' and associated pairs of points P and P' onto a plane parallel to the planes of section. The intersection of a suitable axis for vane rotation with this reference plane is a point X on the diagram having the following property: Circular arcs such as PP' and P'P'', centred upon X extending from P, P' etc. with a sense and length corresponding to the required rotation, do not intersect the associated curve or curves (such as RPP'R') on which P, P' etc. lie.

The kinematic problem of the designer is to determine the boundary of the pivotal region on the diagram, occupied by all points such as X. Within this region, a final choice of the axis of vane rotation can then be made on hydrodynamic and constructional grounds. As all the information required to construct the boundary of the pivotal region can be derived from the solid system by projection on to a plane, the kinematic problem of design (as distinct, for example, from the hydrodynamic design problems) now appears as essentially two- rather than three-dimensional. Such problems may be referred to as pseudo three-dimensional.

Limiting Positions of Centres of Rotation for Points on Curve Segments

We now develop in a general manner a method of constructing the boundary of the pivotal region for a given set of contours and associated points projected on to the reference plane.

Let pPVp'P'' in Fig. 3 represent one of the contours formed by the intersection of the cone and a plane parallel to the reference plane, P
and P' being the points of intersection of this contour with the perimeter of the lamina when this occludes the cone. Suppose now that the lamina rotates anticlockwise to open the valve, about some point within pPVp'P'. This centre of rotation cannot lie below PNT, the normal to pPVp'P' at P; P would otherwise move into the region outside the contour - i.e. this point on the perimeter of the lamina would penetrate the surface of the cone during the motion. Similarly, the required centre of rotation cannot lie to the left of P'NT', the normal to the contour at P', if P' is not to move outside the contour as the lamina rotates. It follows that the required centre of rotation cannot lie outside the region bounded by P'NT.

When the lamina has turned through the desired angle, α, we will suppose that its plane (or the straight line through the points P and P' on its perimeter) now cuts the contour in p and p'. Then P and P' will have moved into positions on the straight line pp'. The circular arc traced by P in the course of this motion about the undetermined centre cannot terminate to the left of p on pp'; so the centre of motion cannot lie below ptw, the normal to pPVp'P' at p.

Similar reasoning can be applied to every intermediate point on the circular arc traced out by P during the required rotation. It therefore follows that the required centre must lie above all the normals to the contour at points between P and p. If the contour is a hyperbola (as where the required axis is parallel to the base of the cone) or a parabola, its radius of curvature increases monotonically from P to p and the normals at these points lie below their envelope, i.e. below the segment Tt of the evolute VTt, the locus of the centres of curvature of the branch pPV of the contour. It then follows that the centre of rotation lies above this envelope; and from this and the previous result we conclude that the required centre of rotation cannot lie outside P'NTtw.

The arc traced by P' during the required rotation cannot terminate above p', so the centre of rotation cannot lie to the left of p't'. But we have already seen that it cannot lie to the left of P'T'. Hence, if the intersection of p't' and P'T' lies to the left of the boundary of the pivotal region - as it can be shown to do when the vane axis is parallel to the base of the cone, to which the plane of pPVp'P' is accordingly perpendicular - it follows that the location of this boundary is uninfluenced by the position to which P' moves when the lamina turns through the required angle. This conclusion is unaltered when P' falls to the left of V. As a corollary, we deduce that the angle through which the lamina can turn about an axis in the pivotal region is not restricted by the motion of P', the path of which will always lie within the cone for any rotation permitted by P.

The boundary of the pivotal region for a rotation of the entire lamina and all its peripheral points through the angle α is now obtained on the plane diagram as the envelope of all such curves as P'NTtw.
derived from the entire set of contours such as pPVp'P' passing through points such as P and P' on the perimeter of the lamina, together with their normals at such points. The outermost of this set of contours will be a pair of straight lines representing projections of the generators of the cone; the innermost contour will be that formed by a plane cutting the cone and grazing the lamina, for which the points corresponding to P and P' coincide.

The argument so far developed has assumed centres of rotation wholly within the contours of the cone. For this reason the treatment is valid only within a region bounded by the arc P'w of the contour of Fig. 3, together with the line P'NTtw. In terms of the complete diagram for the entire lamina, we have to establish separately the boundaries of that part of the pivotal region lying outside the innermost contour.

Inspection of Fig. 3 shows that there do indeed exist points such as E, external to pPVp'P'w, about which PP' can rotate while remaining wholly within the contour. This situation can be examined with the aid of Fig. 4. Clearly, the rotation of P about some point to the right of, and wholly outside, the contour pPVp'w presents no new features, since P lies on a curve segment having the centre of rotation on its concave side exactly as in the case discussed previously. The line below which the axis may not be located corresponds to tw produced, as before; or, if the required rotation brings the intersection of the lamina with the contour far below p, so that t moves far outside the contour, the evolute VTtw'v itself becomes this boundary. On the other hand, the upper boundary, determined by the requirements of the motion of P', is fixed by quite different considerations.

If the lamina is supposed to rotate anti-clockwise, as before, P' must move in the same sense as P, i.e. downwards in Fig. 4, if its centre of motion is to the right of VP'w'w. Its movement may be limited by the intersection of its arc of travel with the contour in such points as p', p'' or p''' The dashed curve P'E'E''E''' is part of the locus of centres of rotation about which the angular movement of P' is restricted to the required angle α by this limitation. A centre of rotation located above P'E'E''E''' will not allow the lamina to rotate through the requisite angle α. The required axis must be located below this line, therefore, and above w'w. On the complete diagram for the entire lamina, the envelopes of all these curves, corresponding to the complete set of contours, define the boundaries of that part of the pivotal region lying outside the innermost contour.

The locus P'E'E''E''' may be drawn on the diagram using the following mechanical construction. A triangular plate is prepared having two adjacent sides including the required angle α. These sides are marked off in scales of equal divisions. One of them is laid against the point P' with one of the numbered divisions coinciding with P'. The plate is then rotated about P' until the intersection of the contour with the
other side occurs at the corresponding division on its scale. The position of the vertex is then marked on the diagram, and the operation is repeated for other pairs of scale graduations. The locus of the vertex positions is the required curve.

Results for a Practical Case

In the standardised design of valve in regular use in Edinburgh, the plane of the vane perimeter is parallel to the axis of rotation and this axis is in turn parallel to the base of the housing cone. In this case, the contours of the cone are hyperbolas and their points of intersection with the vane plane lie on a straight line. The envelope of the normals to the complete set of hyperbolas at their points of intersection with the vane plane is then

\[ y = - \tan^2 \phi \cdot \tan \theta \cdot x \pm 2\sqrt{(h-b)\tan^2 \phi \cdot \sec^2 \phi \cdot \tan \theta \cdot x - (h-b) \tan^2 \phi + b} \]

- where the symbols have the meanings shown in Fig. 2.

Fig. 5 shows the innermost and outermost contours and the trace of the vane plane for the standard Edinburgh valve, for which \( \phi = \tan^{-1}0.1 \) and \( \theta = 25^\circ \). The lower branch of the envelope of the normals, also shown on this diagram, lies in this instance far above all such points as T in Fig. 3, the points of tangency of these normals with their corresponding evolutes, in the region of practical interest bounded by the generator of the cone. This corresponds to a situation in which T is far to the right of the position shown in Fig. 3 for each contour of the complete set, so that in practice the envelope of all such normals as PNT defines the lower boundary of the pivotal region. In this case, consequently, the boundary of the pivotal region can be determined without reference to the position of p.

The part of the upper boundary of the pivotal region outside the innermost contour has been constructed for a turning angle \( \alpha = 55^\circ \) by the mechanical method described above. The upper boundary for zero angle of rotation, also shown in Fig. 5, is simply the upper branch of the envelope of the normals to the hyperbolic contours.

Two features of Fig. 5 are specially noteworthy:

(a) The pivotal region is of finite extent in the y direction except at the cone axis. At any specified distance from the cone axis, therefore, there is a multiplicity of axes about which the vane can rotate in the required manner. It follows that, for any given axis of rotation displaced from the centre of the cone, there is a set of parallel plane laminae which will rotate in the same manner as that shown.

In other words, so long as the pivot is displaced from the cone axis,
a vane of finite thickness is not limited to line-contact with the cone but may seat upon it in a zone of finite width all round its perimeter. This fact is of great practical importance, for in consequence the valve is readily made leak-tight and the broad sealing surfaces (which do not slide or rub against each other) are less vulnerable to accidental damage or wear than a line-seal.

(b) The axis of rotation cannot lie between the cone apex and the sealing plane of the vane. If this axis is embedded in a vane of appreciable thickness, that part of the vane lying on the 'wrong' side of the axis will foul the cone as the vane rotates. In the design adopted, in which the offset of the vane axis is between 0.15 and 0.25 of the local radius of the housing, this fouling takes place in a region of the vane near the pivots and the extremities of the minor axis. Controlled removal of material from the 'back' edge of the vane in this region (which of course lies clear of the sealing zone) allows the vane to open to any desired angle, at which its motion is positively limited without the provision of extraneous projecting 'stops'.

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FIG. 1
FIG. 3

FIG. 5


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Introduction

Some years ago the departments of Chemical Engineering and Medicine at the University of Edinburgh formed a team to collaborate on the design of an artificial heart, capable of maintaining the blood circulation in place of a diseased or damaged natural heart for substantial periods of time. No such apparatus is at present available; existing heart-lung machines and other known devices for artificially maintaining the circulation are traumatic to blood, seriously degrading it in various ways in the course of a few hours. An atraumatic blood pump, capable of maintaining the circulation of a living human subject for periods of days or weeks under hospital conditions, would be of great clinical value in cases where the patient's own heart required a prolonged period of rest or treatment. Moreover, the development of such a pump would be an advance of critical importance towards the ultimate goal of a totally implantable self-contained permanent substitute for the natural heart.

The degree to which blood is damaged by artificial pumps, valves and other foreign components introduced into the circulatory system is apparently governed both by the chemical nature of the materials of construction and by hydrodynamic factors. At present, the chief aim of the Edinburgh Group is to investigate the hydrodynamic variables. The pursuit of this aim requires the provision of a test facility in which the degree of blood trauma caused by pulsatile pumps of various hydraulic designs can be compared, both in vitro and in animal experiments. Such pump comparisons must be made under similar conditions of output; and the latter must be matched to the largely undetermined physiological requirements of the organism whose circulation is to be maintained. We therefore require a driving mechanism, or actuator, capable of operating whatever kind of pulsatile blood pump is under test in whatever manner proves best suited to the proper maintenance of the animal circulation.

This paper describes such an actuator, capable of driving any suitable pulsatile blood pump and allowing effectively independent and positive control, during operation, of three pumping variables, viz. stroke frequency (pulse rate), stroke volume and pulse shape (displacement/time characteristic).

Existing Actuators

Mechanical pulse generators designed to drive experimental blood pumps are well known; some models, indeed, are now commercially available. With the possible exception of an electronically controlled electro-hydraulic actuator tested at Rice University some years ago, all such machines known to the author are pressure-pulsers, rather than
positive-displacement devices. In these, a reservoir of air or hydraulic fluid, maintained at a controlled pressure by means of blowers or pumps, is connected at recurrent and controllable intervals through mechanically or electrically operated valves with the driving cylinder or diaphragm chamber of the blood pump, which consequently ejects blood into the arterial side of the circulatory system. The driving fluid is then exhausted for a controlled period through other automatic valves in the remainder of the cycle, and the blood chamber of the pump refills from the venous side of the blood circuit.

In such a system the pumping characteristics are necessarily interdependent, and it is correspondingly difficult to determine experimentally the physiological effects of any single one in isolation. For instance, the volume of blood ejected from the pump at each stroke depends upon the duration of the connection with the high-pressure reservoir and upon the balance between the pressure of the driving fluid and such resistances as the stiffness, friction and inertia of the moving parts of the pump, the inertia of the fluid contents of the blood chamber, and the impedance of the blood circuit. Most of the resistive factors are dependent on time-derivatives of the motion, so that any change of pulse rate simultaneously affects the stroke volume - as does any change in the pulse shape, or relative duration of the ejection phase of the pumping cycle.

Mechanical Principles of the Present Actuator

The actuator now to be described, designed to overcome these difficulties, is a positive displacement hydraulic pulse generator which can be coupled to the blood-pump under test in the manner shown diagrammatically in Fig. 1. A fixed quantity of incompressible driving fluid, normally water, completely fills the working space between the bellows of the actuator, the driving chamber of the blood pump - here represented as a diaphragm pump - and the connecting tubing. The liquid column functions here as a positive inextensible link connecting pump and actuator. The motion generated by a linkage in the latter is thus transmitted faithfully to the pump, apart from attenuation by tube-wall compliance.

The essential features of the actuator itself are shown in Fig. 2. The actuator bellows are compressed between end-plates reciprocated by two coaxial sliding push-rods, each of which carries at its further end a projecting lug or follower moved by the nutation of a single swash plate. The lugs are arranged on opposite sides of the common axis of the push rods and are constrained by guides to move in a fixed plane containing this axis. They bear on opposite faces of the central portion of the swash-plate assembly, and are forced into contact with these faces by a compression spring acting between the bellows end-plates.

The outer portion of the swash plate assembly can tilt about a diametral axis defined by trunnions fixed in the inner surface of a large drum which partly encloses the swash plate. This drum is arranged
to rotate about the principal axis of the machine collinear with that of the push rods and bellows, and is driven by means of a belt from a motor equipped with a variable speed gear. The outer part of the swash-plate assembly is thus made to rotate about the principal axis at a controllable speed which determines the pulse rate. The inner part of the swash plate assembly, whose faces bear against the lugs of the push-rods, does not rotate; it is prevented from doing so by the engagement of a radial slot in its surface with one of the lugs. The inner and outer parts of the swash plate assembly are force-fitted to the inner and outer races respectively of a ball journal, which constrains them to remain collinear while allowing the outer part to rotate freely relative to the inner.

When the swash plate assembly is tilted about the trunnion axis to a fixed angle of inclination with the principal axis of the drive drum, rotation of the latter causes the inner part of the swash plate to nutate. The component of this motion in the plane of the lugs causes the latter and the push rods to which they are fixed to move symmetrically in opposite directions, so compressing the bellows or allowing them to expand under the influence of the internal compression spring acting on the end-plates. When the swash plate is set nearly normal to the principal axis the amplitude of the rotation is very small; when the plate is tilted at a large angle to this position the amplitude of the bellows motion is large. The stroke volume may thus be controlled by varying the angle of tilt of the swash plate.

The attitude of the swash plate is governed by a link, coupled at one end to the plate by trunnions such that the axis of the revolute joint is parallel to the axis of tilt of the plate. The link is coupled at the other end by a spheric joint to the face of a disc which is free to rotate about an axis parallel to, and capable of being set collinear with, the principal axis of the machine. A screw mechanism allows the disc to be retracted or advanced along its axis of rotation. The arrangement of disc, link and swash plate, together with the frame of the machine, essentially constitutes a slider crank chain, the inclination of the swash plate, and hence the stroke of the pump, being regulated by screw adjustment of the position of the slider on the fixed frame member.

When the stroke volume is small, it varies nearly linearly with the displacement of the disc along its axis. As the main motion of the mechanism is rotation in a plane perpendicular to the direction of this motion of adjustment, the latter can readily be made independently of the former, i.e., while the machine is running.

When the axes of disc and swash plate are set collinear the inclination of the swash plate remains constant as it rotates. The linear displacement of the push-rod lugs and bellows then varies sinusoidally with the rotary motion of the driving drum, and the displacement/time characteristic or pulse shape is likewise sinusoidal if the drum rotates at constant angular velocity. When, however, the
disc is located off-centre by a lateral displacement of its pivot along a transverse slot in the supporting frame, the inclination of the swash plate to the axis varies in the course of rotation. The pulse shape accordingly differs from the sinusoidal to an extent dependent on the displacement, adjustment of which thus provides a means of controlling this characteristic of the pump output.

Kinematic Analysis

The conditions under which such a control of pulse shape can be effected with the minimum simultaneous alteration of amplitude (stroke volume) - and, of course, of pulse rate - may be examined in a preliminary way with the aid of Fig. 3. The essential slider crank chain mechanism governing the inclination of the swash plate is shown here in two sets of three configurations, corresponding to three angular positions of the drive drum and swash plate about the principal axis. The two sets of diagrams compare the inclinations of the swash plate at a fixed axial setting of the freely rotating disc for each of three angular stations of the drive drum, (a) when drive drum and freely rotating disc are coaxial, and (b) when their axes are moved out of coincidence (but remain parallel) in the plane of the figure.

In case (b) it is apparent that when, in the course of the rotation of the drum, the axis of tilt of the swash plate sets itself normal to the plane containing the displaced principal- and disc-axes, (as in (i) and (iii) of Fig. 3), the inclination of the swash plate is markedly different, in general, from that attained in case (a), where the principal axis coincides with that of the disc, for any given fixed distance between the latter and the plane of the swash-plate trunnions. On the other hand, comparison of configurations (ii) (a) and (b) shows that when the axis of tilt of the swash plate lies in the plane of the figure, lateral displacement of the disc axis in that plane has relatively little effect on the swash plate angle. This suggests that if the push-rod lugs, or their points of contact with the faces of the swash-plate, lie in a plane containing the principal axis but perpendicular to the plane in which the disc axis is displaced, the stroke or maximum axial travel of the push rods may be little affected by the lateral displacement of the disc. For such an arrangement the pulse shape and amplitude adjustments would be expected to be substantially independent.

This analysis, though useful in a preliminary approach, is incomplete for a fundamental and noteworthy reason: The mechanism is truly three-dimensional, in the sense that no kinematically equivalent plane mechanism can be derived from it by a simple projective transformation. In particular, Fig. 3 does not reveal that, although the movement of the push rod lugs in the mechanism described reaches its maximum extent at (ii) for case (a), the maximum occurs at some other angular position of the drive drum in case (b) - a circumstance which greatly increases the influence of axial displacement on pulse shape. Furthermore, it is not evident from Fig. 3b that displacement of the disc
axis changes the inclination of the link, and hence of the swash plate, in position (ii) as well as in positions (i) and (iii). Both these factors, not readily analysable in terms of Fig. 3, affect the degree to which the pulse shape and amplitude settings interact. The correct choice of design parameters, minimising this interaction while allowing alteration of the pulse characteristics over a substantial range, can only be made by consideration of the three-dimensional equation of motion.

The essential features of the three-dimensional geometry are shown in Fig. 4. The equation of motion of the mechanism is:

\[
y = r \sin \alpha \cot \left[ \sin^{-1} \left( \frac{s}{1} \right) \sin \left[ \cos^{-1} \left( \frac{r^2 + s^2 - x^2}{2rs} \right) \right] \cos \left[ \tan^{-1} \left( \frac{b}{1} \right) \cosec \alpha \right. \right. \\
\left. \left. \sin \left( \alpha + \sin^{-1} \left( \frac{a}{b} \sin \alpha \right) \right) + \tan^{-1} \left( \frac{b}{1} \cosec \alpha \sin \left( \alpha + \sin^{-1} \left( \frac{a}{b} \sin \alpha \right) \right) \right) \right) \right]
\]

The Design Problem and its Computer Aided Solution

The dimensionless parameters whose best values have to be determined are ratios of b, l, s and r. The design problem is to choose the three ratios in such a way that while the required range of pulse-shape variation is covered by the attainable range of adjustment of a, this adjustment has, at the same time, the minimum effect on the stroke volume.

In the present limited state of knowledge of their physiological effects, pulse wave-forms are sufficiently characterised by the ratio of the times during which the pump chamber is ejecting and filling. In a human subject at rest, this ratio is about 1:3; as the level of bodily activity increases it approaches 1:1. These ratios were taken to define the limits of the variation of pulse shape required of the Edinburgh machine.

It was perceived on consideration of the three-dimensional diagram, and confirmed by trials with a preliminary Meccano model, that inasmuch as a cannot exceed r in a practical mechanism, the required range of pulse shapes could only be obtained for s < 1. It was also noticed that unless b > r, the swash plate nutation contains a prominent second harmonic when the disc is displaced from the axis, introducing what is presumably an undesirable 'double-beat' into all non-sinusoidal pulses generated. In a case where b is very small or zero this phenomenon can easily be apprehended with the aid of a two-dimensional diagram similar to Fig. 3b.

Consideration of these constraints greatly simplified the computational search for an optimal choice of the design parameters. The procedure adopted in the original design work was to choose trial values
of $\frac{b}{r}$ and $\frac{g}{r}$ for insertion into the equation of motion, and to compute
curves of bellows displacement versus angular position of the drive
drum for different values of the lateral disc displacement parameter, $a/r$, at each of several settings of the axial position of the disc. The
curves so obtained were examined for subsidiary peaks arising from the
intrusion of harmonics; if satisfactory from this point of view, the
heights and locations of their maxima were recorded as functions of $a/r$,
providing information about the influence of this variable on stroke
volume and pulse shape at each axial setting of the disc. This procedure
was followed over ranges of trial values of $b/r$ and $g/r$. The required
volume of computational work would have been enormous had not the con­
straints on the variables revealed by preliminary analysis limited the
necessary range of investigation. In fact the programming skill of my
colleague, Mr. J. Ponton, allowed the necessary work to be performed on
a PDP 8 machine, a very small computer.

Specimens of the computed curves of bellows displacement versus
drum angle for the design parameters finally chosen are shown in Fig. 5.
For the optimised design it is seen that, at maximum or moderate ampli­
tudes, alteration of $a/r$ from 0 - 0.8, well within the practical range of
adjustment, produces the required threefold change in the ratio of ejection
to filling times, a regular waveform substantially free from cusps, and
a concomitant change in amplitude of only a few per cent. At very small
amplitudes the undesired influence of $a/r$ upon amplitude becomes large
and the pulses develop prominent subsidiary peaks; but these effects are
significant only below the amplitude range of practical importance.

The Existing Machine

The absolute size of the Edinburgh machine, built to the
proportions thus arrived at, was fixed by the physiological requirement
that the maximum displacement of the pump should be about 80 ml. per
stroke against a maximum total head of about 1 atmosphere and by the
cross-sectional area of suitable available bellows. It is compact—a substantial advantage in the crowded conditions of an experimental
operating theatre—occupying (without variable speed gear and driving
motor) a space approximately 35 cm x 18 cm x 15 cm overall. The
apparatus as constructed by Mr. Robert Hardie, engineering technician
of the Group, amply fulfils the design requirements and has been in
regular and intensive use for about eighteen months with relatively
little maintenance.

A Design Improvement

The original concept leading to the adoption of a three-dimensional
design was that the main motion and two required adjustments can all be
made independent of each other by arranging that the three motions are in
mutually perpendicular planes. It is now evident that adjustments to
this mechanism along the mutual: perpendicular axes of a Cartesian
coordinate system are not exactly independent, though they can be made sufficiently so for the present purpose. Subsequent examination of the matter, made by my assistant Mr. C.J. Knight with the aid of the I.B.M. computing package 'Continuous System Modelling Program', has shown that the parametric independence is improved if the adjustments are made along orthogonal paths on a spherical coordinate system, the amplitude adjustment being radial and the pulse shape adjustment azimuthal. In other words, the slotted frame member guiding the lateral motion of the disc should be curved in the plane of the principal axis. The practical advantage of this improvement appears insufficient to justify its incorporation into the existing machine, but might warrant its inclusion in the design of a new one.

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Definitions of Symbols

a - displacement of axis of free-rotating disc from principal axis.
b - effective radius of free-rotating disc.
l - perpendicular distance between planes of rotation of swash plate trunnions and free-rotating disc.
r - distance between axes of swash plate trunnions and revolute joint of link, measured in plane of swash-plate.
s - effective length of link, i.e. distance between spheric and revolute joints.
x - $\sqrt{l^2 + a^2}$.
y - displacement of push rod from trunnion plane along principal axis due to inclination of swash plate, assuming effective radial length of lug = r.
\(\alpha\) - angle turned through by trunnion axis of swash plate from plane of push rod lugs.
Fig. 3
Successive Angular Positions of Swash Plate & Disc, In & Out of Line.

N. MacLeod: A Mechanical Pulse Generator for Driving
Experimental Blood Pumps.

a) Axes Collinear
b) Axes Displaced

\( \alpha = \infty \)

\( \alpha = 45^\circ \)

\( \alpha = 0^\circ \)

FIG. 4
Geometry of Swash Plate Motion.
FIG. 5  Computed Curves of $\gamma' \propto \alpha$ for $\gamma' = 1.5$, $\gamma' = 3.5$, $\gamma' = 3.8$.

BOTTOM
APPENDIX (D)

Estimation of maximum and minimum Wall Shear Stress In
Circulation Loop

In all the hemolytic experiments employing the circulation
loop shown in Figure (3.7), the flow rate has been maintained at
3.3 l/min. The smallest conduit in the circuit is the 1/2 inch
tubing shown in Figure (3.7). For this tubing, the following is
obtained (based on the assumption of steady flow):

- Tube radius = 0.64 cm
- Flow rate = 3.3 l/min.
- Average fluid velocity = \( u = 43 \) cm/sec.
- Re Number (average) = 1820
- Blood density = 1 gm/cm^3
- Blood viscosity = 0.03 poise

The fluid flow is therefore laminar, and the laminar wall shear stress
\( \tau \), is given by (20):

\[ \tau = \frac{u}{r} \]

where \( \tau \) = wall shear stress, \( u \) = average fluid velocity, \( r \) = tube radius.

The fluid shear stress in the turbulent vicinity of the occlusive
valves cannot be deduced theoretically (20). The wall shear stress
at the surfaces of the occlusive valves cannot also be deduced theoretical
without making very wide assumptions. The following estimate of \( \tau \)
is based on the assumption that the occlusive vane of the valve
is maintained flat during the flow. The flow is also assumed to be steady.

\[ \tau = f \frac{u^2}{2} \]

where \( f \) = friction factor. If \( f \) is taken to be 1.328/Re^{0.5} (see ref. 20)
and assuming Re = 1820, \( u = 43 \) cm/sec. then \( \tau \) = 29 dynes/cm^2.

N.B. The maximum wall shear stress, estimated above, is based
on the following wide assumptions: (i) the vanes are maintained
flat, which does not coincide with their observed behaviour during
the flow, and (ii) the flow has been assumed steady; in the circuit,
the flow is pulsatile.

During part of the flow cycle, the blood in certain parts of
the circuit (e.g. the atrial reservoir), is almost stationary.
Consequently the minimum fluid \( \rho \) and wall shear stress levels in
the circuit is almost zero.
2. Maximum and Minimum Shear Stress Levels

It is reasonable to assume that the sites of highest shear stress are at the valves of the pump. Here the valve vanes constitute mid-stream obstructions, such as occur nowhere else in the flow system. During the initial part, at least, of the period in which a valve is opening, the vane is at a large angle of incidence to the stream and constitutes a gross obstruction to the flow. High rates of shear might then be expected in the wake of the vane. During this opening phase of the vane motion, however, the fluid velocity is at first low, and the wake shear-stresses are therefore likely to be quite small also; and as the flow rate through the valve increases, the angle of incidence of the vane diminishes tending to reduce the size and intensity of the wake. A well-designed vane ultimately adopts a position well aligned with the flow, when the wake associated with its streamlined form is very small. At some intermediate phase of the opening cycle the degree of bluff-body disturbance, corresponding to some instantaneous combination of increasing flow and diminishing angle of incidence, presumably passes through a maximum. But it is evidently not possible to calculate the corresponding transient value of the shear stress, nor even to estimate a priori whether this local instantaneous maximum stress does in fact exceed the shear stresses at other sites in the flow system – for instance, at the vane leading edge.

The observation that the degree of haemolysis depends strongly on the angle to which the vane opens does suggest that haemolysis occurs mainly in the wake, and that this is accordingly the region at which the shear stress attains an absolute maximum, at least in those experiments in which the angle of vane opening was limited by stops; for in no other region of the flow would the shear stress levels be sensitive to vane orientation at relatively low angles of incidence. It therefore follows from the foregoing that it is not possible to make any reliable estimate of the maximum shear stress attained in the system, nor to correlate this with the degree of haemolysis observed, for comparison with, e.g., the data of Blackshear (Ref. 17).

It seems likely, in any case, that the haemolytic phenomena reported here, which are strongly influenced by the nature of the surface, have little to do with those discussed in Ref. 17, in which no systematic consideration is given to the influence of the nature of the material of the solid wall. In this connection it is noteworthy that the maximum value of the shear stress in the wake of the vane would be expected to correspond to a velocity gradient of order of magnitude

\[
\frac{\text{Max. fluid velocity}}{\text{Radius of curvature of downstream edge of vane}}
\]

Taking the maximum fluid velocity as of the order 100 cm/sec. and the radius of the sharpest edge of the vane as \(10^{-2}\) cm., the maximum possible value for the velocity gradient in the wake would appear to be of order of magnitude \(10^4\) sec\(^{-1}\). This is an order of magnitude below the/
the threshold proposed in Fig. 14 of Ref. 17 for haemolysis in steady flow in tubes, and is probably far below that required to generate the turbulent shear stress of 50,000 dynes/cm² quoted in Ref. 17 as the lethal level for red cells at short exposure times. This again suggests that the effects observed in the present experiments are different from those reviewed in Ref. 17.

Blackshear (17) has drawn attention to the haemolytic effects associated with rates of shear below a certain minimum, quoted as $10^3$ cm/sec for certain kinds of surface. The shear rates in the present experiments were always below this value almost everywhere in the flow system, and were of course periodically zero in those parts of the system adjacent to the valves when the latter were closed. The fact that nevertheless little haemolysis occurred in the system as a whole relative to that at the valves may be attributed to the atraumatic nature of the walls of the flow passages.
Resistive and Reactive Components of the Adopted Model of Circulation

The input impedance of the mock arterial loop shown in Figure (3.7), is given in Figure (3.9). The impedance of the system may be reduced to its resistive and reactive terms thus:

\[
\text{Res}_i = Z_i \cos \psi_i \\
\text{Rea}_i = Z_i \sin \psi_i
\]

\(\text{Res}_i\) = resistive component of impedance \(Z_i\)
\(\text{Rea}_i\) = reactive component of impedance \(Z_i\)
\(i\) = frequency
\(\psi_i\) = phase angle at frequency \(i\).

For the circuit, the resistive and reactive components of the impedance spectra are given below. The values are computed from the data collected at the fundamental frequency of 1.1 Hz. These values have been confined to the first six harmonics, which contain the bulk of the flow (see chapter 3). For these harmonics the system has been assumed to be linear.

<table>
<thead>
<tr>
<th>Harmonic</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>1.0</td>
<td>2.2</td>
<td>3.3</td>
<td>4.4</td>
<td>5.5</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>(Z_i^*)</td>
<td>1235</td>
<td>470</td>
<td>490</td>
<td>590</td>
<td>1000</td>
<td>1670</td>
<td>200</td>
</tr>
<tr>
<td>(\text{Res}_i^*)</td>
<td>1235</td>
<td>465</td>
<td>489</td>
<td>572</td>
<td>870</td>
<td>568</td>
<td>34</td>
</tr>
<tr>
<td>(\text{Rea}_i^*)</td>
<td>0</td>
<td>56</td>
<td>-25</td>
<td>-153</td>
<td>-500</td>
<td>1570</td>
<td>196</td>
</tr>
<tr>
<td>(\psi_i) (degrees)</td>
<td>0</td>
<td>8</td>
<td>-3</td>
<td>-15</td>
<td>-30</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>

\* units: gm. cm. sec^{-1}

The inductive and capacitive components of \(\text{Rea}_i\) cannot theoretically be obtained; the inductive and capacitive components of the model however, may be obtained experimentally. The absolute values of the resistive, capacitive and inductive components of the input impedance have no bearing on the conducted hemolytic studies and consequently have not been individually determined.

The dynamic characteristics of the model have already been described on pages 49-51 and in Figures 3.9-11.
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Declaration

I hereby declare that the thesis presented here is of my own composition and that the work described herein is my own.

M.A. Alami.