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METHODS FOR THE ASSESSMENT OF THE EFFECTS OF DRUGS ON THE ARTERIAL SYSTEM IN MAN

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The arterial system is an anatomical entity which serves a number of circulatory functions. Therefore if we wish to describe the effects of drugs on the system it is first necessary to define the precise functions we are concerned with and devise methods which are appropriate for their measurement. The arterial system has four main functions.

Firstly, it has a 'damping' function whereby it converts the large pressure fluctuations generated by the ventricles (ventricular pressure) into the relatively steady pressure head (arterial pressure) needed to provide continuous perfusion of the tissues.

Secondly, the arterial vessels act as a rapid transport system which permits the blood to be distributed at high velocity and low energy cost from the heart to the tissues.

Thirdly, the arterial system contains control mechanisms which tend to hold mean arterial pressure within closely defined set values. Any deviation of the pressure from these values initiates responses which tend to drive the pressure back towards the set values. Thus the tissue perfusion pressure is maintained within fairly narrow limits.

Fourthly, the smaller vessels of the arterial system especially the arterioles offer about two thirds of the total resistance offered by the circulation to the flow of blood. The resistance offered by these vessels is capable of being varied by nervous, chemical, physical or other means. Thus in the constant pressure system which the arterial system is, the vessels responsible for the bulk of the resistance act as taps regulating the flow of blood to the various tissues in accordance with their needs.

Assessment of arterial damping

The proximal arterial tree, with its high content of elastic tissue, is mainly responsible for this function. Energy stored in the distensible tissues during systole is fed back into the system during diastole thus limiting the rise in arterial systolic pressure and the fall in arterial diastolic pressure. The pressure fluctuation or *pulse pressure* of 120/0 seen in the left ventricle is converted into one of 120/80 in the brachial artery, i.e., it is reduced by two thirds. The ability to 'damp' or reduce the pulse pressure depends on the stiffness of the arterial wall. Arterial stiffness increases with distance from the heart. Thus, pulse pressure tends to be greater in distal arteries than in proximal arteries. Stiffness also increases with age so that pulse pressure tends to be low in the distensible arterial systems of infants and high in the stiffened arteries of the aged.

Though the effect of drugs on arterial stiffness could be estimated by their effect on pulse pressure, the usefulness of the measurement is limited since a number of other factors, other than wall stiffness, affect pulse pressure. These other factors include the stroke output and the peripheral resistance which affect the systolic rise and the diastolic fall in pressure respectively. Most drugs which would influence arterial stiffness would also affect cardiac output or peripheral resistance.

A better index of wall stiffness is *arterial pulse velocity*. As the arterial wall stiffens from childhood to old age, arterial pulse velocity increases from about 4 to about 10 m/s. Thus, if one records arterial pressure in the brachial and radial arteries simultaneously with indwelling needles and measures the distance between the needles and the pulse transit times, arterial pulse velocity can be calculated. Pulse velocity can also be measured without puncturing arteries. If pneumatic cuffs are inflated on proximal and distal parts of a limb at a pressure somewhere between diastolic and systolic arterial pressure, the pulse will cause a fluctuation in cuff pressure as it passes each cuff. Mechanotransducers can also be placed over arteries where they come close to the limb surface to record the time at which the pulse passes the observation point. Both these non-invasive methods allow measurement of the pulse transit time and the arterial length needed to calculate arterial pulse velocity.

Assessment of arterial transport

The large arteries have such a wide bore that they offer virtually no resistance to blood flow. Thus blood can be transported from the aorta to the brachial artery with a fall in mean arterial pressure that is barely detectable. This is useful in that it permits blood to be transported to the tissues at low energy cost. In addition the total cross-sectional area of the proximal arterial tree is so small compared with other parts of the circulatory system that the blood is transported through the arterial tree at high velocity.

These facts have implications if we wish to study the effects of drugs on the larger arteries. In general,

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drugs alter the calibre of arteries by contracting or relaxing the smooth muscle in their walls. Since the resistance offered by the major arteries is barely measurable, dilatation of the major arteries, no matter how great, will not decrease the total resistance offered by the circulatory system by a measurable amount and thus will have no appreciable effect on the flow through the arteries. In addition, since the resistance offered by large arteries is so small, an arterial vasoconstriction which doubled arterial resistance would again have virtually no effect on flow since again there would be virtually no change in the total resistance offered by the circulatory system as a whole. It is only when a major artery is almost occluded by spasm or obstruction that the resistance that it offers rises sufficiently to alter flow.

There are a number of methods available to measure changes in arterial calibre which do not depend on resistance and flow measurements. One of the best known of these is *angiography*. Though this involves an invasive technique and the hazards of injecting a radio-opaque substance into a circulatory area which may be at risk, it does permit the calibre of the arteries to be assessed and is particularly useful where gross changes in calibre have been induced. However it probably has a limited role to play in clinical pharmacology.

A method which has a greater potential for clinical pharmacology is *induction angiometry* (Kolin, 1978). This is a system of sensing vascular diameter based on electromagnetic induction. Loop sensors are introduced via an intravascular angiographic catheter by the Seldinger technique into the artery to be studied. An electromotive force is induced by an alternating magnetic field in a resilient lens-shaped fine wire loop which lies in the artery so that the loop width is equal to the vessel diameter as it varies in the course of vasodilatation or constriction. The primary magnetic field is generated by an insulated wire loop which is congruant to a secondary loop to which it is linked in the fashion of a bifilar wire pair. With change in arterial diameter, the primary and secondary loops change in diameter so that the mutual inductance of the loop-shaped coreless transformer varies. The resulting e.m.f. is a logarithmic function of vessel diameter. The system is calibrated by pulling the loop through a channel of step-wise varying width. Diameter changes of the order of a micrometer can be recorded in arteries of about 5 mm internal diameter.

A non-invasive method which can give qualitative information about changes in the diameter of arteries involves the use of the *Dopler shift ultrasonic technique* (Franklin, Schlegel & Watson, 1963; Light, 1977; Parker, 1977). When placed over an artery this can detect changes in flow velocity. Since blood velocity in arteries is an inverse function of diameter over a certain range of diameters, this technique allows estimation of diameter change. The principle of the technique depends on the change in frequency of an ultrasound wave as it is reflected back towards the sending direction by the moving particles in a moving fluid. The sound transmitted to particles moving away from the sound source will be reflected back as a wave of lower frequency, the decrease in frequency being a function of the velocity at which the particles are moving.

In vitro methods of assessing the effects of drugs on arteries have been used extensively but the relevance of these studies to the *in vivo* effects of drugs in man may be questioned. Human arteries have been removed post mortem from fingers and other convenient tissues or from umbilical cords and used for pharmacological purposes. Isolated vessels may be perfused at constant flow and the effect of drugs estimated by the changes in inflow pressure that dilatation or constriction may cause (De la Lande & Rand, 1965). In addition mechanotransducers may be used to measure the effect of drugs on the length of strips or the perimeter of rings of isolated arteries (Bohr, 1973).

Assessment of arterial pressure regulation

Methods have been described which permits the investigator to assess the ability of the arterial system to maintain arterial pressure within narrow limits (Bristow, Brown, Cunningham, Goode, Howson & Sleight, 1971). Basically these methods test the effectiveness of the baroreceptor reflexes. Arterial blood pressure is raised or lowered by pharmacological or physiological means and the resulting changes in the interval between heart beats (measured from a ECG record) are plotted against changes in arterial pressure. Changes in the slope of the resulting curve indicate changes in the sensitivity of the baroreceptor reflexes and hence the ability to regulate arterial pressure.

The sensitivity of the baroreceptor reflexes tends to fall with increasing age (Randall, Esler, Bullock, Maisel, Ellis, Zweifler & Julius, 1976) and postural hypotension becomes increasingly common (Collins, Dore, Exton-Smith, Fox, Macdonald & Woodward, 1977).

Assessment of arterial resistance

About two thirds of the total resistance offered by the circulation lies in the small arteries and arterioles. Since resistance varies with the fourth power of the radius of these vessels and the smooth muscle that they contain is very responsive to many pharmacological agents, measurement of the resistance function of the arterial system has played a very prominent role in assessing the effects of drugs on the arterial system.

The resistance offered by the systemic or pulmonary circuit may be measured by using the

hydraulic equivalent of Ohm's law:

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

where R is the vascular resistance, ΔP the pressure drop across the circuit and F the flow round the circuit. The pressure drop can be calculated by measuring mean arterial pressure and assuming that central venous pressure is zero. Mean arterial pressure (MAP) may be estimated from measurements of brachial arterial pressure, assuming that

$$MAP = \frac{1 \text{ systolic} + 2 \text{ diastolic pressures (mm Hg)}}{3}$$

For the pulmonary circuit it is necessary to introduce a catheter into the pulmonary artery to measure the pressure there.

Flow round the circuit may be measured by calculating cardiac output using the Fick principle (Warren, 1948) or an indicator dilution technique (Zierler, 1962).

To measure regional changes in resistance, the same principles may be employed but in many instances it is only necessary to measure regional flow since changes in arterial pressure are minimal or taken into consideration in the design of the experiment.

Another method of measuring regional resistance is to suddenly occlude the artery responsible for perfusing the region under investigation. The rate at which the 'run off' of pressure occurs is a function of the peripheral resistance; if resistance is high the run off will be slow and *vice versa*.

Man is admirably suited for the investigation of drug actions on peripheral resistance blood vessels. This is because he has two symmetrical arms and legs with readily accessible arteries and veins and methods are available for accurate quantitative measurement of blood flow in the limbs.

To make such an investigation, experiments can be carried out along the following lines. The subject lies on a couch and both forearms are inserted into plethysomographs to measure forearm blood flow as described later. One forearm is used as the experimental forearm and the other used as the control. An indwelling needle or catheter is introduced into the brachial artery on the experimental side pointing peripherally. With proper technique and a sharp needle this procedure is quite painless if a small amount of local anaesthetic is injected into the overlying skin. Saline is infused at a constant rate (2–4 ml min^{-1}) into the artery via the catheter by an infusion pump. To make sure that the infusate is being distributed evenly to all the areas of the forearm it is advisable to infuse a dilute histamine solution (1 mg min^{-1}) at the beginning of the experiment. If distribution is even, the skin of the forearm shows a uniform blush; if the infusate is streaming down some branch of the brachial artery, the blush is restricted to a certain area of the forearm skin. In the latter case the catheter should be readjusted until the blush is uniform.

With the plethysmographs replaced in position, blood flow is measured on both sides until the flow rates on the two sides are approximately even. After a suitable control period a switch is made so that a saline solution containing a suitable concentration of the drug under study is infused into the forearm. The switch is made without the subject knowing and even when large changes in blood flow occur, the subject is usually unaware of the change. This is important since peripheral blood flow is influenced quite dramatically by emotional factors (Blair, Glover, Greenfield & Roddie, 1959).

This system has certain advantages. Since the drug is being administered to one forearm only, high concentrations can be delivered to the tissues being investigated while the total body dose remains low. Secondly, the peripheral tissues are extremely potent in inactivating drugs administered to them. Thus 16 μg min^{-1} of noradrenaline have to be administered intra-arterially to mimic the effects of 4 μg min^{-1} administered intravenously (Brick, Hutchison & Roddie, 1967). This means that 75% of the infused drug is inactivated in one passage through the forearm. In addition, the opposite forearm acts as an excellent control for the experimental forearm. Any changes in blood flow due to general systemic changes such as change in blood pressure, release of vasoactive hormones, change of activity in sympathetic vasoconstrictor or vasodilator nerves will occur in both arms so that any difference in the response of the experimental from that of the control forearm must be due to the effect of the drug.

With this system it is easy to carry out experiments to construct dose-response curves for the drug and to see how these curves are affected by antagonists or synergists.

One factor which may make the interpretation of results more difficult is that any change in the rate of blood flow induced by the drug will alter the concentration of the drug in the blood being delivered to the tissues (Lowe & Robinson, 1963). Thus a strong vasoconstriction which results in a gross reduction in blood flow will, with a constant rate of drug infusion, result in very high concentrations of the drug being presented to the tissues. Similarly a strong vasodilatation will result in a fall in the concentration of the drug in the perfusing blood.

This problem does not arise when the drug is administered intravenously. Here the concentration of the drug in the blood arriving at the forearm will remain the same regardless of the rate of blood flow in the forearm. However, with intravenous infusion, the advantage of having a meaningful control forearm is lost and the effect of the drug on the forearm is complicated by any systemic effects that the drug may

have which cause reflex or other changes to occur in forearm blood flow.

Where one is primarily interested in blood flow through skin, *hand plethysmography* is the measurement of choice since blood flow to the hand is primarily to skin. Similarly, if one is interested in muscle blood flow, *forearm plethysmography* is the appropriate method since forearm blood flow is primarily to muscle. If one wishes to eliminate the skin component of forearm blood flow it may be necessary to suppress blood flow to the skin by the iontophoresis of adrenaline into this tissue (Barcroft, Bonnar, Edholm & Effron, 1943).

Though intra-arterial infusion is the most effective method of administration of a drug to peripheral tissues in man, drugs may also be administered by iontophoresis into the tissues or by subcutaneous or intramuscular injection. These methods are less useful since it is more difficult to be certain about the amount of drug reaching the tissues, it is difficult to achieve steady-state conditions and the injections or iontophoresis may cause damage or irritation to the tissues and thus affect local blood flow.

Methods of measurement of peripheral blood flow

An attempt has been made in Table 1 to give a 'consumer' type comparison of methods available for measuring blood flow in the human limb. The methods have been arranged in descending order of precision, from quantitative to qualitative methods. 'Convenience for subject' means the absence or otherwise of unpleasant features such as needle puncture and the need to lie still for a considerable time. 'Simplicity for observer' is related to the need for elaborate equipment and special skills. Greenfield, Whitney & Mowbray (1963) have provided a detailed and extensively-referenced evaluation of the various methods.

In assessing drug effects, where observations on a number of patients are to be collated, a precise quantitative method is likely to be required. *Venous occlusion plethysmography*, widely used for some decades, has the edge over other methods in this respect. In addition it has the advantage of being non-invasive and fairly convenient in use, though some experience is needed to gain the required degree of

Table 1 An assessment of various methods of estimating blood flow in the limbs. A star grading is used (* — poor, **** — very good) for a rough comparison of the methods under several headings

Method	*Precision*	*Convenience for subject*	*Simplicity for observer*
1. plethysmography (water-filled)	****	***	**
2. plethysmography (strain guage)	***	***	***
3. indicator dilution	***	*	*
4. indicator clearance	**	**	**
5. external calorimetry	**	***	**
6. skin temperature	**	****	****
7. pulsation volume	*	***	**
8. ultrasound (Doppler)	*	****	***

References

1. Greenfield (1960a); Greenfield *et al.* (1963).
2. Whitney, 1953; Greenfield *et al.* (1963).
3. Zierler (1962).
4. Kety (1949).
5. Greenfield (1960b).
6. Diji & Greenfield (1960).
7. Burton (1939).
8. Franklin *et al.* (1963); Light (1977); Parker (1977).

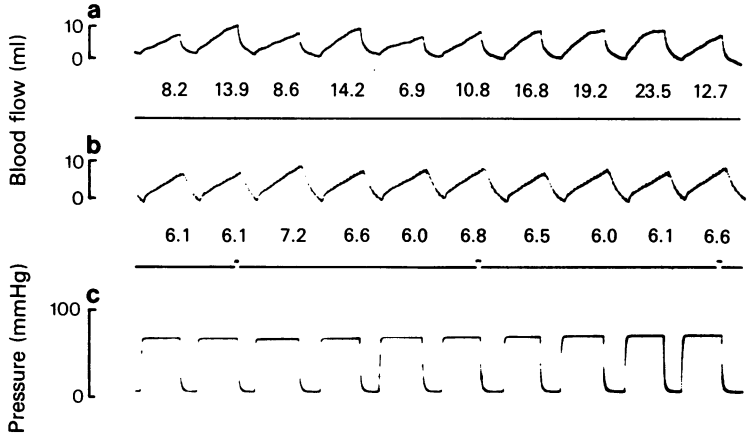

Figure 1 Typical plethysmograms of blood flow in the right hand (a) and left forearm (b) under stable resting conditions. Pressure in the collecting cuffs is indicated at (c). Rates of blood flow measured from the slope of the record (after the initial inflation artifact) are given below the traces in ml (of blood) 100 ml^{-1} (of tissue) min^{-1}. Time signals, between (b) and (c), are at 1 min intervals.

skill. Briefly, as its name implies (Greek plethysmos = increases), a plethysmograph is a device for measuring the rate of increase in volume of a limb segment when a pressure cuff is applied proximally so that, for a short time, arterial blood can enter the part while venous blood cannot leave. Further details of the method are given later.

The *indicator dilution* technique also provides a quantitative method but has the disadvantage of requiring access to both an artery (for indicator injection) and a vein (for sampling). It is also difficult to be sure of adequate mixing and a representative sample. *Indicator clearance* involves measuring the rate of removal of an indicator (e.g., radioactive sodium) from an injection site; it does not give a quantitative measure of total blood flow to a region.

External calorimetry and measurement of *skin temperature* depend on the fact that skin temperature in an extremity is strongly influenced by local blood flow. The methods do not provide accurate quantitative information on blood flow but can afford a sensitive index of changes in skin blood flow rate. Measurement of skin temperature has the advantage of extreme simplicity; modern electronic thermometers with multiple thermojunction leads give direct readings of temperature at a number of sites in a matter of seconds.

Although in certain carefully defined circumstances measurements of *pulsation* volume may give a qualitative indication of changes in blood flow, at worst, the method may be highly fallacious. Rate of flow and pulsation volume do not necessarily change in parallel, an extreme case being that pulsation may be transmitted to a limb in the absence of flow!

Use of ultrasound and the Doppler principle can give an indication of flow velocity in a major blood vessel but estimations of absolute flow can not be made unless the vessel diameter is measured simultaneously. The method is not applicable to measurement of diffuse flow in a part.

Since *venous occlusion plethysmography* is unique in providing a non-invasive method for measuring blood flow directly and quantitatively, it will now be described in some detail.

Limb segments which may be studied include digits, hands, feet, forearms and calves. The upper limbs are rather more convenient to study than the lower limbs. The hands and the forearms have circulations which are functionally quite different and this influences the results obtained. The hands consist mainly of skin and bone. Bone blood flow tends to be constant. Flow in the skin of the hands is determined largely by thermoregulatory and baroregulatory requirements. It is also influenced by local skin temperature and fluctuates quite markedly even under apparently stable conditions (Figure 1a).

The forearm contains a considerable proportion of muscle. Forearm blood flow is therefore influenced by muscle activity as well as by thermoregulatory and baroreceptor reflex activity. Again local skin temperature has an important influence on blood flow. In contrast to hand blood flow, forearm blood flow is remarkably steady under stable resting conditions (Figure 1b). Because of this, the forearm is often chosen as the site for the investigation of the effects of drugs on the peripheral circulation.

Measurement of volume changes in a segment can be made directly using displacement of water or air, or

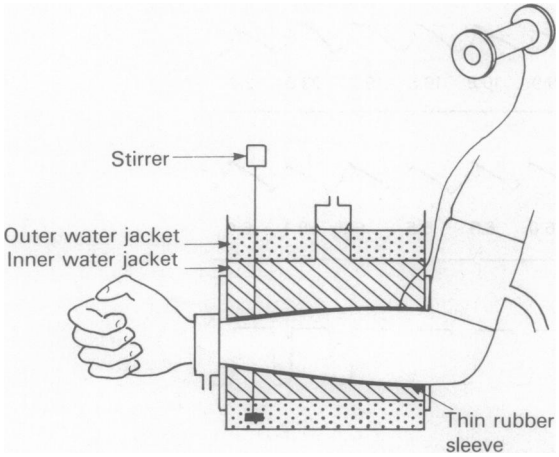

Figure 2 Principle of the water-filled plethysmograph.

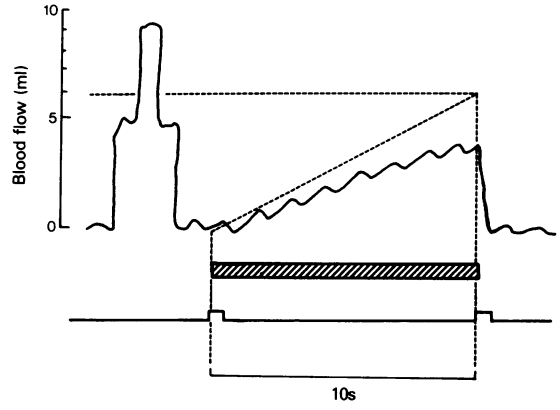

Figure 3 Schematic diagram of the interpretation of a plethysmogram. Venous occlusion is applied for 10 s as indicated by the hatched panel. The initial rate of rise in forearm volume (arterial inflow rate) is 6 ml in 10 s.

may be estimated from change in circumference at one or more points. Change in circumference may be deduced from change in resistance of a thin column of mercury within a rubber tube surrounding the part (mercury in rubber strain gauge). This method is simpler and more convenient in some respects than the water-filled plethysmograph but is less precise.

The principle of the water-filled plethysmograph is shown in Figure 2. The segment of forearm to be studied is inserted into a thin rubber sleeve which is then mounted in a water jacket in which displacement of water can be accurately recorded. This inner water jacket is surrounded by an outer water jacket which is stirred to maintain a constant temperature, say 35°C, a comfortable temperature for forearm skin. In fact, one of the big advantages of the water-filled plethysmograph is its ability to maintain a constant forearm skin temperature thereby avoiding fluctuations in blood flow from this source. The stirring motor is driven by low voltage electricity, say 6–12 v, just in case there is an accident and the electric current is conveyed to the subject via the plethysmograph water!

'Empty' veins are required at the onset of each individual measurement, since the impounded blood must be accommodated in the venous system. For this reason the limb is placed a little above heart level so that the veins are in the collapsed state.

Venous occlusion is provided by an upper arm cuff rapidly filled (e.g., from a compressed air cylinder) with air to a pressure a little below diastolic arterial pressure, say 60–70 mm Hg. By inflating the cuff for 10 s and deflating for 5, the blood flow rate is measured 4 times per min (Figure 1). To avoid error due to venous return from the hand, a cuff at supra-systolic pressure is inflated at the wrist a minute or so before measurements are begun. This cuff is kept inflated during measurement of forearm blood flow; 15–20 min of cuff inflation can be tolerated without discomfort. If blood flow measurements are to be made over a prolonged period, the wrist cuff must be released at intervals.

An exacting requirement of the method is that the subject must lie almost motionless for fairly long periods. Opening or closing the hand, or turning the head from side to side causes unacceptable movement of the part within the plethysmograph, leading to distortion of the record. For the same reason, measurements cannot be made during exercise of the forearm muscles, although the recovery phase of hyperaemia can be recorded.

The *recording system* must provide a continuous record of forearm volume versus time. Changes in water level in the funnel of the plethysmograph can be measured directly, but a convenient method is to connect the air above the column of water to a float recorder or pressure transducer.

The *record* obtained (plethysmogram) is shown schematically in Figure 3. The initial calibration is produced by injecting and then withdrawing two 5 ml volumes of water by syringe through a connexion to the inner water jacket. When venous occlusion is applied, forearm volume increases as blood accumulates in the venous system. The rate of increase is initially linear and then tends to fall off as venous capacity is exceeded. The rate of arterial inflow is represented by the initial rate of rise which may be obtained by joining corresponding points (e.g., peaks) on such arterial pulse waves as lie in a straight line.

Provided that calibration of the system and paper speed are constant, blood flow is directly proportional

to the initial slope of the plethysmogram. It is often useful to express flow as the rate per 100 ml of tissue. The volume of tissue within the plethysmograph and subsequently measuring volume by displacement in a graduated container. A simple apparatus may be used (Greenfield *et al.*, 1963) to measure the slopes of plethysmograms rapidly, the results being expressed as ml blood flow 100 ml^{-1} tissue min^{-1}.

As stated, the technique requires good cooperation by the subject and considerable skill in the observer. The help of an experienced technician in maintaining the equipment and assisting during measurements is invaluable. Further details of the recording technique and of the interpretation of records are given by Greenfield (1960a) and Greenfield *et al.* (1963).

Validation of the method is also discussed by the above authors. Briefly, the following points have been demonstrated in man (Wilkins & Bradley, 1946; Formal & Doyle, 1957; Wallace, 1958):

1. Distal arterial pressure is not affected by application of the pneumatic collecting cuff.
2. There is no leak of venous blood past the collecting cuff for a finite time after the pressure has been applied.
3. The rising venous pressure does not initially reduce the rate of arterial inflow.
4. The impounded blood causes the segment under study to swell proportionately to the rate of arterial inflow, without significant displacement of tissue or body fluids from the plethysmograph.
5. The hydrostatic pressure of the water in the inner jacket and funnel has no appreciable effect on the flow recorded in the range of heights normally used.

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