A SIMPLE WATER-FILLED PLETHYSMOMOGRAPH FOR MEASUREMENT OF LIMB BLOOD FLOW IN HUMANS

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Fundamental principles underpinning the study of cardiovascular physiology can be emphasized by measuring blood flow. Plethysmography is an appropriate, noninvasive technique to use but may not be available to some institutions. Therefore, for measurement of blood flow in human limbs, we developed a simple water-filled plethysmograph that may be built with minimal technical support. The device is formed from a plastic cylinder and houses a latex sleeve sealed at either end by means of circular flanges and rubber O-ring seals. Limb volume changes are transcribed using an air-filled piston recorder. This instrument proves to be sensitive and accurately determines limb volume changes over time. Utilizing an appropriate venous occlusion protocol, predicted vascular responses to postural challenge and physical exercise may be followed. In response to a questionnaire, a majority of students (n = 33) agreed that performing blood flow measurements succeeded in relating theory to practice, improved technical and observational skills, and made the learning experience real. This modified plethysmograph proves to be a valuable teaching tool in human physiology classes.

Key words: human physiology; noninvasive; cardiovascular; plethysmography

Measurement of blood flow provides valuable information about function and regulation of the circulation. Blood flow (Q) is dependent on the pressure difference (ΔP) between any two given points in the circulation, i.e., between the arteries and veins, and resistance (R) to the flow of blood according to the equation Q = ΔP/R. Therefore, measurements of blood flow can reflect changes in cardiac output, mediated by heart rate and/or stroke volume, although in some circumstances regional blood flow may change independently of any alteration in cardiac output. Blood flow measurements also provide information about the caliber of regional arterioles indicative of vasomotor tone. Such physiological changes are rapidly evoked in response to a variety of stresses, including physical exercise, drugs, changing posture, and local or systemic heating and cooling, that may be used to examine homeostatic control mechanisms. Recognizing the potential value of performing blood flow measurements as an aid to the understanding of cardiovascular physiology, we set out to make a suitable measurement tool available to the students in our University.

The problem addressed in this communication concerns the availability of an appropriate instrument for noninvasive measurement of blood flow in human limbs. The most common method used for determination of blood flow in humans is venous occlusion plethysmography (explained in Brief theory of venous occlusion plethysmography), a technique used by physiologists for close to a century (5, 11, 13).
Because the technique of plethysmography is noninvasive, it is an attractive measurement tool for use in the physiology teaching laboratory. The plethysmograph is essentially a volume recorder that detects subtle changes in the volume of an organ over time. Although traditionally used for measurement of blood flow (12–14, 16, 18) plethysmographs may also be employed for determination of respiratory movements (4). Whitney (18) developed the very effective mercury-in-Silastic strain gauge plethysmograph to measure changes in limb circumference. Any change in limb circumference is proportional to change in blood volume and therefore reflects rate of arterial blood flow during occlusion of limb veins. This method continues to be used widely by research scientists and has greatly contributed to our understanding of the mechanisms of blood flow regulation (12). Although extremely effective and easy to use, strain gauge plethysmographs are rather expensive per unit. This reduces the possibility of students performing individual measurements in the practical setting.

Early plethysmographs consisted of either water-filled (8) or air-filled compartments in which the limb segment was sealed. These devices also have their respective advantages and disadvantages. Changes in air volume are influenced by body temperature, therefore requiring a correction factor to enable accurate volume determination. Alternatively, water-filled devices may remove this confounding influence, but there is then a need to maintain stable water temperature, given the influence this may have on blood flow to the limb (1). Nevertheless, water-filled and air-filled plethysmographs accurately determine change in volume of the entire limb segment, allowing measurement of arterial blood flow and other physiological variables. This ability of the water plethysmograph to provide an integrated value for blood flow is in contrast to the strain gauge method, where blood flow values are calculated from changes in circumference of the limb under investigation. The accuracy in blood flow measurements yielded by water plethysmographs and strain gauge plethysmographs is, however, not quantitatively different (15).

Because water-filled plethysmographs may also be used to examine the influence of temperature perturbation on blood flow, we chose to explore this method in the present communication. Water-filled plethysmographs were traditionally constructed from glass (5, 11) or brass and other metals (1, 8) to form either conical or boxed-shaped designs. By use of these materials, some of which are no longer appropriate, the water-filled plethysmograph would prove difficult to manufacture in great numbers. We are not aware of any reference to the design of a simple water-filled plethysmograph that may be used in the teaching laboratory. Recognizing the need for such an instrument, we developed a simple yet affordable device with a high degree of performance for the measurement of limb blood flow in humans. This modified plethysmograph may be relatively simply constructed by a university or college workshop and has proved to be a valuable teaching tool in our human physiology classes.

METHODS

Brief theory of venous occlusion plethysmography. The method of venous occlusion plethysmography utilizes the capacitance function of the veins. With regard to circulatory function, this itself is an interesting and relevant observation for students to make. By applying pressure sufficient to collapse the limb veins but not the arteries, outflow of blood from the veins is temporarily checked, whereas arterial blood flow into the limb continues unhindered for a short time. As a consequence, the limb swells in direct proportion to the rate of arterial inflow of blood, and this volume change is detected by the plethysmograph. Measurements are confined only to the linear portion of the volume record when there is a constant rate of arterial inflow (see Fig. 1C). With continued occlusion, venous volume and pressure rise, the arterial-venous pressure difference narrows, and consequently there is a reduction in rate of arterial inflow, indicated by a leveling of the volume record. The volume record is also influenced by blood escaping under the cuff as venous pressure approaches that in the cuff. Greenfield and Patterson (9) have presented a more detailed description of circulatory events that take place during occlusion of forearm veins.

Design of the water-filled plethysmograph. The main modification of the plethysmograph presented here is the use of a plastic cylinder to form the main
body or reservoir of the device. Other plethysmographs have utilized conical or boxed-shaped designs formed mainly from metals. We have also introduced a new strategy of using different-size circular flanges at wrist end and forearm end of the device. These serve to taper the latex sleeve around the forearm inside the instrument. Overall, this new design dramatically reduces construction time and also limits the number of joints that must be water tight. There are only two joints located at either end of the device between the main body (cylinder) of the plethysmograph and the latex rubber sleeve that accepts the forearm. The method used to seal each end of the plethysmograph was essentially the same as that used by other authors (see next section).

**Construction of the water-filled plethysmograph.** A 180-mm length of unplasticized polyvinyl chloride (UPVC) tubing with a diameter of 110 mm was first cut to form the main body of the plethysmograph. This was actually a section of domestic drain pipe. Three holes were drilled into this cylinder to locate 1) a mercury thermometer and rubber bung (13 mm diameter, centered 20 mm from the wrist end), 2) the barrel of a 20-ml syringe (end removed) into which was inserted a rubber bung and outlet tubing that led to the piston recorder and writing arm mechanism (21 mm diameter, centered 55 mm from the wrist end), and 3) a rubber bung and tubing connected to a 50-ml calibrating syringe (13 mm diameter, located 50 mm from the forearm end at an angle ~110°–120° from the vertical) (Fig 2).

To ensure that the latex sleeve was tapered to fit closely around the enclosed forearm, the opening of
Simple water-filled plethysmograph that utilizes a cylindrical rather than a traditional box-shaped design. Only a small volume of water is required to fill the device, and this together with the insulating unplasticized polyvinyl chloride (UPVC) material of the main cylinder body reduces the rate of heat loss from the water. The various individual components of the plethysmograph are clearly labeled and described in the text: a) main cylinder body, b) outer circular flange, c) inner circular flange, d) bolts and wing nuts placed equidistant around the circumference, e) rubber O-ring seal, f) thermometer and rubber bung, g) tubing leading to calibrating syringe, h) 20-ml syringe barrel acting as water outlet, i) latex rubber sleeve, and j) wooden support stand. (An economical cork-float recorder is shown here, although in our experience an air-filled piston recorder is more user friendly).
the plethysmograph at the wrist end was made smaller than the forearm-end. At the wrist end, two circular flanges (UPVC or similar plastic), external diameter of 110 mm and internal diameter of 83 mm, were first cut and then drilled (or threaded) with 12 5-mm holes centred 6 mm from the outer edge. These holes were positioned equidistant around the circumference of the flange. For the inner flange that was adhered to the cylinder body, we have found it easier to thread the holes to accept 25-mm lengths of 5-mm-diameter steel studding (without a screw head). This was necessary as the clearance to accept a bolt head inside the cylinder body was limited by the need to allow space for locating the rubber O-ring seal. The outer circular flange was drilled with >5-mm holes to allow easy positioning before being clamped down. At the forearm end, an identical procedure was followed. These flanges were of external diameter 140 mm and internal diameter 110 mm. Sixteen holes centered 10 mm from the outside edge of the flange were drilled around the circumference to accept 5-mm bolts. Given the greater circumference of this flange (Fig. 2) at the forearm end, the bolts were located outside the main cylinder body.

The inner circular flanges at wrist end and forearm end were subsequently cemented to the main cylinder body (Polypipe Solvent Cement, Doncaster, UK). These joints were thoroughly coated with standard silicone sealant to prevent water leakage when the plethysmograph is filled. Rubber O-rings made from lengths of rubber cord (diameter 5 mm) were then adhered to the inner rim of each flange. Finally, the inner sleeve of the plethysmograph was made from latex rubber sheeting of length 230 mm and width 300 mm. This was cut and glued (Pritt Copydex, Winsford, UK) along its length to form a cylinder to accept the forearm. When the latex sleeve and rubber O-rings are sandwiched between the two circular flanges, a water-tight seal is formed at each end of the plethysmograph. The outer flanges were secured in place using wing nuts around the circumference to compress the rubber seal of the plethysmograph.

Blood flow measurement procedure. The procedure outlined here provides good quality results. However, depending on the resources available, our experience suggests that a number of simplifications to the method can be made and still allow collection of meaningful data in the teaching laboratory. These modifications to the procedure are highlighted in parentheses. Before any experiments were conducted, all of the procedures were formally approved by the Ethics Committee of Liverpool John Moores University.

The plethysmograph cylinder was passed over the arm of the subject and allowed to rest on the wooden base. Foam blocks were placed under the hand and elbow to ensure that the arm was centered in the device. A small occluding cuff was then placed over the wrist (may be eliminated), and a larger collecting cuff with a hose of 10 mm internal diameter was positioned around the upper arm. The collecting cuff was connected through 10-mm hosing to a pressure reservoir via a three-way tap that opened both to air and to the reservoir. Before measurements were taken, using a foot or bicycle pump the reservoir was pressurized, and this was indicated by a mercury manometer. To achieve venous occlusion, the pressure was chosen to fall between estimated venous pressure and diastolic arterial pressure, typically 50 mmHg. A standard blood pressure cuff and manometer will suffice, although a less rapid occlusion of the forearm veins will be achieved (see Fig 2). This arrangement ensures that the collecting cuff is rapidly inflated and allows definition of a distinct point of onset for the record of forearm blood flow. The plethysmograph was subsequently filled with 34-35°C water through the opening where the thermometer is later placed. Water occupies the space between the cylinder wall and the latex sleeve but does not come into contact with the forearm itself. The hydrostatic pressure secures the latex sleeve around the forearm, thereby allowing detection of a limb volume change. In our experience, after the device appears to be filled, we have found it best to add yet more water by using the calibrating syringe. It appears that, in the absence of this additional filling, the latex sheet exhibits some compliance, which may be a potential source of error. However, if this filling procedure is followed, it seems there is no need to apply any padding around the circumference of the wrist or the forearm where there may be a very slight bulging of the latex sleeve.

Transduction of the volume change in the plethysmograph to the kymograph drum was achieved using a
A standard piston recorder and lever arm mechanism. A pocket of air was left in the tubing between the water level and the piston itself. To calibrate the device, 1- to 2-ml volumes of water were injected into the plethysmograph with the limb in position and the displacement of the lever arm recorded. This was repeated over a range of 10–15 ml, and the linear slope was later calculated in millimeters per milliliter. One minute before the assessment of blood flow began, and throughout measurement, the occluding cuff to the hand was inflated above systolic arterial pressure (\( \approx 200 \text{ mmHg} \)). Forearm blood flow (FBF) measurements were then recorded in accordance with the following cycle: 10-s inflation of the collecting cuff (50 mmHg) followed by a period of 10-s cuff deflation. This cycle was repeated for a total of 3 min, generating a total of nine measurements of blood flow, three per minute. The occluding cuff to the hand was then deflated for \( \approx 3 \) min before another cycle of blood flow measurements continued. Depending on the individual protocol of the experiment and the frequency of measurements required, the resting interval between cycles may be extended.

By use of sphygmomanometry, arterial blood pressure [from which mean arterial pressure (MAP) was derived] was recorded before the measurements of blood flow began. Forearm vascular resistance (FVR) was later calculated according to the equation

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FVR = \frac{\text{MAP}}{\text{FBF}}.
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**Calculation of forearm blood flow.** From the volume record captured on the kymograph drum recorder, the linear slope of the relationship between vertical displacement and time was calculated (mm/s) (Fig. 1). The volume record was then converted to milliliters per second using the calibration value. Finally, to express blood flow as a percentage change in tissue volume in units of milliliters per 100 ml of tissue per minute, the total change in limb volume was expressed as a percentage of the total forearm volume. To perform this final step in the calculation, the volume of the segment of the forearm enclosed in the plethysmograph was measured. This was determined by displacement of water from a large measuring cylinder, hand volume was subtracted from the volume of the entire limb.

**RESULTS**

As shown in Fig. 1, the water-filled plethysmograph successfully detected a linear increase in forearm volume upon occlusion of the veins draining the arm. The individual arterial pulsations are also visible when the lever arm mechanism is set at an appropriate degree of sensitivity. FBF was greater if recorded in the supine (2.97 ml \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}, SD = 0.41) compared with the sitting position (1.04 ml \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}, SD = 0.12). The lower rate of change in limb volume over time for the seated position is clearly visible in Fig. 1B.

A marked postexercise hyperemia was evident after 60 s of maximal isometric handgrip exercise (Fig. 3). Peak blood flow recorded in the forearm 1 min after exercise was 19.2 ml \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}. There was a concomitant reduction in FVR after exercise, indicative of relaxation of the arteriolar smooth muscle (Fig. 1C). Followed through the recovery period,
FBF returned toward the baseline but still remained elevated at 10 min after exercise (FBF = 5.4 ml \cdot 100 ml tissue$^{-1}$ \cdot min$^{-1}$; Fig. 3).

Water temperature in the plethysmograph was monitored frequently throughout the experiment and cooled by only 1°C from 34–33°C over a 90-min period. The laboratory temperature remained constant at 26.5°C throughout the experiment.

**DISCUSSION**

Our objective was to incorporate an appropriate practical cardiovascular physiology class into the curriculum at our University. We therefore designed and developed a simple water-filled plethysmograph for measurement of blood flow in human limbs. After building and testing a prototype device, our University workshop machined (using a lathe) and drilled the circular flanges for 12 additional plethysmographs. This work, together with cutting and drilling of cylinder bodies, is much less labor intensive if it is performed by a workshop. Actual construction of the device, including cementing of rubber O-rings and fitting of the latex sleeve, was completed by the authors with the assistance of laboratory technical staff. This instrument may therefore be built with a little support from a standard college workshop.

**Applications to teaching cardiovascular physiology.** With respect to processes of teaching and learning, a number of fundamental physiological principles may be emphasized using the technique of limb plethysmography. These include the following observations. 1) Blood flows from regions of high- to low-pressure occlusion of the veins draining the limb leads to an increase in both venous volume and pressure, thus demonstrating that arterial blood is under a higher circulatory pressure. 2) Regional inflow and outflow of blood are not always equal—this is the main principle underlying venous occlusion plethysmography and demonstrates the capacitance function of veins. In this regard, a connection may also be made between increase in venous filling and rise in venous pressure that accompany postural change from the supine to the standing position. 3) Blood volume and blood pressure are intimately linked—during the period of occlusion there is a corresponding rise in venous volume and venous pressure (not measured). Evidence for this is clear, because as occlusion time continues, the rate of limb volume change declines as a consequence of, among other factors, blood now under a higher pressure escaping from the arm under the collecting cuff (See Fig. 1C).

**Performance of the plethysmograph.** Recognition of the fundamental principles described above may be made, together with realization of the major aims of the practical class. We developed the plethysmograph to evaluate responses of forearm blood flow to postural challenge and to physical exercise. The intent was for students to investigate neural and local metabolic regulation of predominantly skeletal muscle blood flow. From results presented, it is clear that this instrument has the capability to detect predicted changes in blood flow that accompany a postural maneuver (Fig. 1, A and B), together with more marked responses seen after physical exercise (Fig. 3). It is not difficult to perform muscle contractions with the forearm enclosed in the plethysmograph, and this allows measurements to continue immediately after the period of exercise. A number of students at our University have also utilized the water-filled plethysmograph to perform undergraduate research projects. Indeed, using water as a medium for displacement lends itself to studies concerned with temperature perturbation that are more difficult to conduct using mercury-in-Silastic strain gauge plethysmography or air plethysmography. One example of such a study is to examine the influence of local temperature changes on predominantly skin blood flow in different body positions.

Although perhaps used more conventionally for the measurement of arterial blood flow, the technique of limb plethysmography has many more applications for both research and teaching. Recent advances include the capability to noninvasively measure venous pressure (3), venous compliance (3, 10, 17), and microvascular fluid filtration flux and capacity (2, 6). Other variables, such as venous outflow and systolic arterial pressure, may also be determined plethysmographically. Although the full potential of the instrument described here has not yet been fully investigated, we can see no reason why such measurements may not be made using the water-filled plethysmograph when appropriate experimental protocols are followed.
Evaluation of the experimental technique in the context of learning. The laboratory practical exercise is a vital component in the process of developing an understanding of scientific inquiry (7). A number of other advantages may also be recognized, including acquisition of technical skills and problem solving abilities, opportunity for students to learn at their own pace, development of a capacity for independent learning, and ability to relate theory to practice (7). Having used the new plethysmograph to develop an appropriate cardiovascular physiology practical class, we were interested to see how students (n = 33) evaluated the learning experience. In response to a customized questionnaire (score 1 = strongly disagree, score 5 = strongly agree), the majority of students either agreed (score 4) or strongly agreed (score 5) that the practical 1) succeeded in relating theory to practice (59%), 2) encouraged evaluative and critical thinking (58%), 3) developed the capacity for independent problem solving and learning (59%), 4) improved technical and observational skills (60%), and 5) made the learning experience real (60%). A number of the neutral responses received were related to difficulties understanding the material as laid out in the module handbook. Given the nature of the questions asked in the practical session, it appeared that some students had not prepared adequately by reading the supplied material beforehand.

Limitations of the plethysmograph and areas of potential improvement. There are limitations to all experimental techniques and the one presented here is no exception. The main limitation of our simple plethysmograph design is lack of a mechanism to control water temperature, which is known to influence forearm blood flow measurements (1). Other investigators have built plethysmographs complete with an external water bath into which cold or warm water is added without affecting the volume of water in the plethysmograph proper (8). This design would, however, complicate the construction procedure tremendously and make it difficult to produce the device in any great numbers. There was, in fact, a minimal amount of heat lost (1°C) from the water in our plethysmograph, presumably due to the small volume of water required to fill the device [~1,000 ml compared with 1,200–1,500 ml reported by Greenfield (8)] and the insulating property of the plastic. The temperature of the enclosed limb will itself serve to maintain the water temperature. It is anticipated that the majority of experiments could be completed before substantial cooling of the water occurred. Alternatively, the plethysmograph could be drained through the side tubing and quickly refilled, allowing experiments to continue.

Unstable hydrostatic pressure as a consequence of fluid moving in or out of the reservoir has been identified as a potential source of error when recording organ volume changes plethysmographically. Measurement error is greatest when compliance of the organ is high and when a slow rate of volume change is being studied over a long period of time, minutes rather than seconds (19). We have not measured the change in hydrostatic pressure in this new plethysmograph during occlusion of the forearm veins and cannot determine the effect this may have on blood flow. Changes in hydrostatic pressure are expected to be small using an occlusion period of 10 s, although this will ultimately depend on the rate of flow into the limb and the volume of water displaced.

In conclusion, using this simple plethysmograph design as an alternative to other commercially available equipment, we have found it possible to implement a practical class that students otherwise would not have an opportunity to experience. The instrument proves to be robust, accurate, and inexpensive to manufacture in numbers with the assistance of a standard University workshop. Students evaluated use of the plethysmograph positively as an aid to learning some of the fundamental principles of cardiovascular physiology.

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