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A New Method of Plethysmometry.

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The method herein described is designed to eliminate some disadvantages of the standard plethysmograph, particularly the necessity of enclosing the object to be measured in a water-tight container through a tightly-fitting rubber cuff.* The new procedure makes it possible to determine volume changes in an open container. This is of particular interest in the clinical determination of blood flow through extremities where speed and ease with which the measurement can be performed are very desirable. The use of an open vessel offers an easy access to the extremity during the measurement and makes it possible to remove and reimmerse it within a short time, which is of advantage in experimental work.

The apparatus to be described can easily be assembled from standard parts available in any physiological laboratory. It requires a balance and a container large enough for the immersion of the object to be measured.

The principle upon which this method is based can be considered a supplement to the principle of Archimedes. According to Archimedes, an object immersed in a vessel of water loses weight equal to the weight of the volume of water which it displaces. Now it can be shown quite easily that the converse is also true namely, exactly the same amount of weight as lost by the immersed object is gained by the vessel in which it is immersed. The reason for this gain in weight is easily explainable as follows: In Fig. 1 we have a solid cylinder, C, immersed in a beaker of water, B, to the level, L. To simplify our considerations we may visualize in the liquid an imaginary rigid U-tube, as indicated in the dotted lines, having a cross section equal to that of the cylinder, C. It is evident that the liquid in our U-tube, up to the level L is in perfect balance. At level L the left arm of the tube is plugged by the lower surface of the cylinder C so that the column of water of the height h in the right arm of the U-tube presses upward on the lower face of the cylinder C with a force F_1, which is obviously equal to h times the cross section of the U-tube which product is in turn equal to the immersed volume of the cylinder between level L and the surface of the water, Ws. This

* For a discussion of the difficulties encountered with the standard plethysmograph, see Abramson, Zazeela, and Marrus, *Am. Heart J.*, 1939, **17**, 194.

force is represented by the vector F_1 and is identical with the buoyancy of Archimedes. Now, according to Newton's third law, there must be a second force F_2 equal to and opposing F_1 acting downward on the beaker of water so that it would apparently gain weight equal to that which the immersed cylinder loses. It is this force, F_2, which is utilized in the method presented here.†

Let us now imagine the beaker of water placed on one side of a balance and a human extremity partially immersed in it, but rigidly supported from the outside so as to avoid any contact with the moving parts of the balance, (as shown in Fig. 2). After establishing equilibrium we allow the part of the extremity immersed to increase in volume by an increment n cc, for instance by occlusion of venous return. This will disturb the equilibrium of the balance and we shall have to add n cc to a beaker of water on the opposite pan of the balance to restore equilibrium and bring the index pointer back to zero. This determination of the amount of water added to

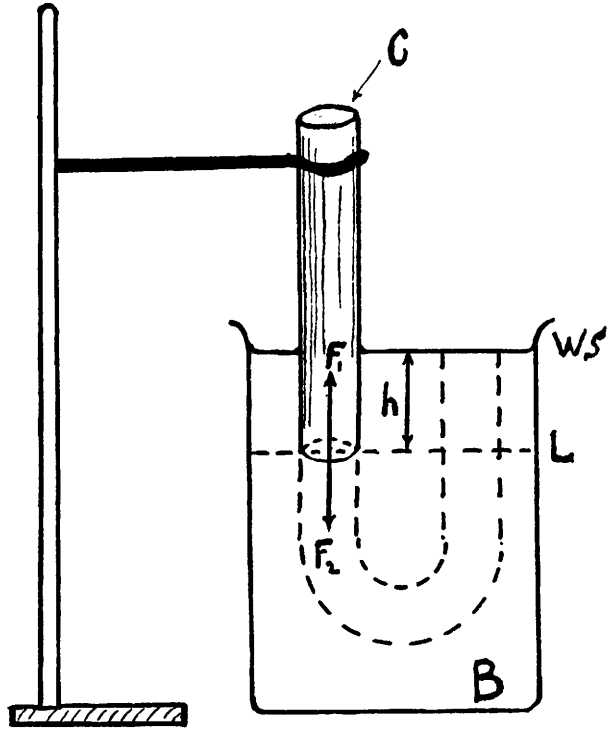

FIG. 1.
Discussed in text.

† The above conclusions hold good for any arbitrarily shaped object immersed.

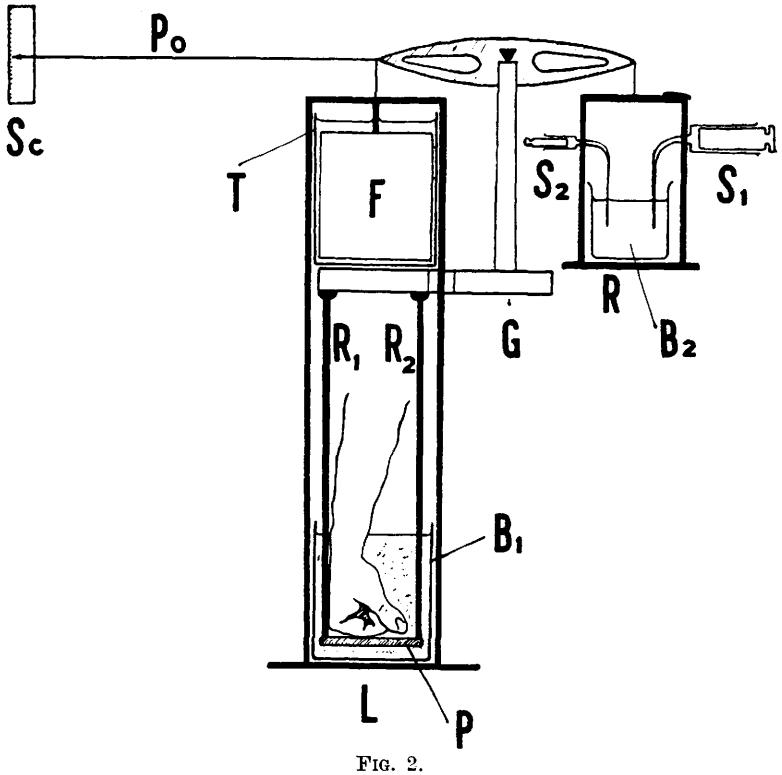

FIG. 2.
Scheme of the Plethysmometer.
Sc: Scale. Po: Pointer. S_1, S_2: Syringes. B_1, B_2: Beakers. F: Float. T: Buoyancy tank. R_1, R_2: Support rods. G: Base plate. L, R: Balance pans. P: Platform.

balance the scale constitutes the measure of the expansion of the extremity.

In the case of small volume changes it is also practical to read them off directly from the deflection of the pointer, after calibration by the addition of a known volume of water from the syringe S_2 (Fig. 2).

Figure 2 shows schematically the set-up used. The essential parts are the beaker of water, B_1, resting on the left balance pan, L, and the beaker of water, B_2, resting on the right balance pan, R. The arm is shown immersed in water in the beaker, B_1, being supported on a platform, P, which is rigidly attached to the ground plate, G, by means of stiff rods, R_1 and R_2. The arm is allowed to lean against R_1 and can, if desired, be rested against an additional support at the elbow for better fixation of position. An adequate weight is placed on the right pan, R, to maintain equilibrium of the balance. The finer adjustment of balance is accomplished by withdrawing or adding water by means of a large syringe, S_1. The small syringe, S_2, is

calibrated in cc and is used to measure the amount of water required to restore equilibrium during the experiment, which amount measures directly the increase in volume of the arm when venous flow is obstructed. To increase the sensitivity we attached a long pointer, Po, the deflections of which were read on the vertical scale, Sc, or could also be recorded on a kymograph drum, if necessary.

For our purpose we needed a balance sensitive to about 0.05 g but strong enough to be loaded with 3.5 kg. By a simple means we made it possible to use a balance originally designed to carry a maximum weight of 1 kg. An air-filled float, F, of approximately the same volume as the beaker, B_1, was attached to the left side of the balance and immersed in a container of water, T, which was supported on the ground plate, G.‡ Its buoyancy just about compensated for the weight of the water in beaker, B_1. The addition of a small volume of water in beaker, B_2, was sufficient to balance the scale.

Even with an infinitely sensitive balance the sensitivity of our plethysmograph is limited by the following fact: If the immersed part of our object gains n cc in volume, the balance begins to descend on that side so that a part of the object will thus emerge from the water. Now, obviously, the motion of the balance in that direction could not continue beyond the point where n cc emerge from the water, since, in this new position of the balance, the same volume is submerged in water as before the expansion of the object; hence the balance ought to be again in equilibrium, if the restoring torque is negligible. Practically the latter is not the case and the deflection is somewhat smaller than the ideal maximum deflection. From this consideration it is clear that the amount of deflection per cc of volume change is inversely proportional to the cross section of the object at water level.

Due to the inertia of the system, our balance does not follow the rapid volume variations associated with the cardiac cycle, but is adequate for recording of variations which are slow as compared with the period of the balance. (4.3 sec in the example cited below.)

The effect of temperature variations on the density of water is negligible in the calibration.

To give an idea of the order of magnitude of the sensitivity of our set-up, we shall cite figures which were obtained in one typical arrangement. With a cylinder of 28 cm^2 cross section the deflection due to 1 cc volume change amounted to .9 mm. Since one cannot

‡ Glycerol can be added to the water in T to obtain aperiodic damping of the balance.

estimate the deflection on the scale closer than 0.1 mm, this constitutes the limit of resolution which corresponds to about 0.1 cc. The sensitivity could, of course, be easily increased by choosing a longer pointer or by reflecting a beam of light from a mirror mounted at the balance pivot.

We have made comparative measurements of blood flow through the hand using this method and the standard plethysmograph. The results were found to check well.

The author wishes to thank Dr. Zazeela and Mr. Marrus for their active coöperation in the physiological tests of the method.

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On the Toxicity of "Q" Substance from *Eberthella typhosa.*

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Bruce White[1, 2] described an acid-alcohol-soluble "protein", extracted from *Eberthella typhosa* and other enteric bacilli, the presence of which was necessary for agglutination of specifically sensitized R bacilli. This substance, which he called "Q" substance, was antigenic but not species-specific. Anti-"Q" sera agglutinated p-forms of *Salmonella* bacilli; R-forms were agglutinated more slowly and to a lesser titer. S-forms contained "Q" but were inagglutinable by "Q" antisera. "Q" antisera gave heavy precipitates with solutions of "Q" substance. "Q" substance also gave a precipitate with normal serum but the specificity of the reaction with immune serum could be demonstrated by suitable dilution of the antigen. The "Q" substance could be differentiated from the P substances extracted by Furth and Landsteiner[3] by means of hot 75% alcohol. White reported "Q" substance as being non-toxic for rabbits, but the dosage was not stated.

E. typhosa, strain Ty 2, was grown on sodium-chloride infusion-agar, pH 7.4-7.6, for 24 hours at 37°C. The growth was washed from the agar with 3-5 cc of saline per Roux bottle. The bacilli were killed by the addition of one volume of pure acetone at 37°C for one hour. The killed flocculated bacilli were collected by centri-

1 White, P. Bruce, *J. Path. and Bact.*, 1932, **35**, 77.

2 White, P. Bruce, *J. Path. and Bact.*, 1934, **39**, 529.

3 Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.