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SHORT COMMUNICATION

Digitalization of a Pressure-Transducer Based Plethysmograph through a PowerLab Equipment for Recording Rat Paw Volume

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ABSTRACT

We presented a plethysmograph built from an old pressure transducer associated with a mercury column. The paw under study was immersed into a mercury vessel for making the measurements. The resultant upward force exerted by the paw was equal to the weight of the fluid that the paw displaced. Since the force exerted equals to the product of the pressure times the area, and the area of the pressure sensor was constant, then the force was directly proportional to the pressure. The accuracy of the measured volume was dependent on the precision of the sensor. In this case, the sensitivity was 50 to 300 µV per Volt, per 10 mm of mercury. This plethysmograph was tested by immersing objects with different volumes and shapes. Results showed a very good linearity. The values recorded were coupled to a PowerLab model 4/30, allowed us to digitalize the values obtained.

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INTRODUCTION

Plethysmographs are built by using different principles. For example, to mention a few, Shejawal *et al*. (2014) measured the volume change of two water-filled columns connected in parallel, one for immersing the paw and the other for measuring the weight change of the water displaced by the paw. The force necessary to insert a rat paw into a mercury column and then the weight developed measured with an electronic balance. Kulkarni *et al*. (2011) utilized a conventional mercury plethysmometer associated to a buzzer that alerts when the mercury level reaches a certain level predetermined by standards of known volumes. Finally, there are also commercial devices; one is from Ugo Basile (www.ugobasile.com). Regarding the possible mercury toxicity to the rat or the researcher, we followed the recommendations of the ATSDR Chemical Specific Health Consultation (Anonymous, 2012). In summary, we designed and tested a novel plethysmograph based on a pressure transducer obtained from old devices and modernized by using a PowerLab equipment for digitalization. This device, besides its use in research inflammation, can be useful for veterinary studies of pro-inflammatory substances affecting farm animals (Jain *et al*., 1995).

MATERIALS AND METHODS

Fig. 1 shows the complete scheme of the plethysmograph. This was built with parts readily available on any Lab. It consisted of a syringe of 50 ml cut at the height point of 30 ml and filled with 23 ml of Hg. Then connected to a three-way valve associated to a 3 ml syringe filled with distilled water to get rid of air bubbles. The pressure transducer was a Stathman's Model P23 (Statham Medical Instruments, Inc; Hato Rey, Puerto Rico), connected to a Fritz Hellige & Co. recording system (Hellige GMBH; D-78 Freiburg in Breisgau, West Germany), and the analog output sent to an AD Instruments PowerLab 4/30, (AD Instruments Proprietary Limited Company, Australia) analyzed with the Chart v5.2 program (eDAQ Inc. Colorado Springs, USA. Software for Windows 2000, XP). The resultant voltage produced by the paw immersion was measured in triplicate, and the mean constitutes the usable value. Then a conversion was done between paw volume and the resultant voltage as shown in Fig. 2.

Animals: The plethysmograph was tested by using normal adult male Sprague-Dawley rats weighing between 250-350 g. The animals were obtained from the vivarium of the Faculty of Medicine of the University of Chile and kept under a light-dark cycle of 12/12 hours,

with food and water *ad libitum*. All experiments were performed by the ethical guidelines of the International Association of the Study of Pain and the Committee on Bioethics of the University of Santiago of Chile, Chile.

Linearity tests: To test the volume measurements, we used a linear regression curve. The equation was: f(x) = **a**+**b**X_i, were f(x) represent the estimated or predicted Y axis values for the observation X_i; **a** represents the intercept of the curve, **b** the regression slope and **r** the linear correlation coefficient. In this case, **a**= 2.43; **b**= 17.66 and **r**= 0.999.

Plethysmograph testing: Before submitting the rat paw to the plethysmograph, a line with a permanent marker was done around the right paw ankle to replicate the measurements. The idea was to immerse the paw in the mercury up to the line. Furthermore, to avoid any movement that the rat could perform, the animals were anesthetized with a brief exposure to a mixture of 2% isofluorane in 100% oxygen. The anesthetic effect lasted no more than 1-2 minutes.

Rat paw egg albumin inflammation: Many irritant agents can be used as inflammation inducers to produce paw edema in rats. One of them is the intraplantar injection of undiluted egg white (50 µL). The egg white induced a non-specific inflammation that lasted for around 6 hours (Chen *et al*., 2015).

Rat paw inflammation testing challenged with ibuprofen: The effect of injected undiluted egg white inflammation and the anti-inflammatory effect of ibuprofen were tested by injecting in the right paw, egg white and/or ibuprofen, according to the following scheme: 1) Three rats were injected with 50 µL of intraplantar egg white and submitted to the plethysmograph to measure paw inflammation at times zero, 60, 120, 180 and 240 minutes; and 2) Three rats were injected with 50 µL of intraplantar egg white and simultaneously with intraperitoneal (i.p.) ibuprofen applied at time zero with concentrations of 16 mg/kg. In a different experiment, three rats with ibuprofen at 40 mg/kg. Then, the resultant inflammation was tested at times zero, 60, 120, 180 and 240 minutes.

Statistical analysis: The results were taken as an average of three measurements and expressed as Mean ± SE.

RESULTS AND DISCUSSION

To see the goodness of fit between known volumes placed into the plethysmograph and the resultant voltage delivered by the Chart program, we tested it in the range from 0.5 to 6.0 mL (Fig. 2). These values are in the range of the rat paw volume of around 1.5 to 4 mL, from normal to inflamed paw for rats weighing 250-350 g. To evaluate the *in vivo* performance of the plethysmograph, the inflammatory effect of intraplantar egg white and ibuprofen were done. Fig. 3 shows the inflammatory effect of egg white on the rat paw and the anti-inflammatory effect of ibuprofen for two doses.

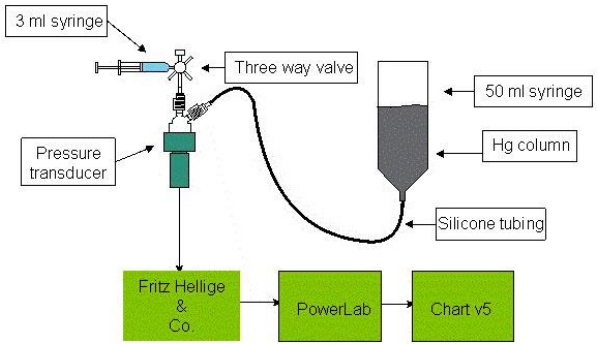

Fig. 1: The figure shows a schema of the plethysmograph. The rat paw is immersed into the Hg column, and the resultant pressure change is sensed by the pressure transducer. This response is sent to the Fritz Hellige device which transforms the pressure into an analog voltage that is fed to the PowerLab digitizer. These changes are visualized by using the Chart v5.2 software.

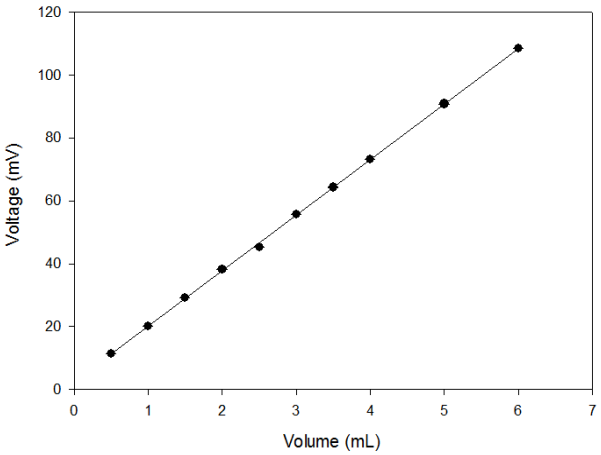

Fig. 2: The figure shows the resultant voltage corresponding to a given volume by immersing different objects with different shapes of a known volume, ranging from 0.5 to 6.0 mL. The regression curve had an r^2=0.999, indicating a very good linearity of the measurements. The experiments were done in triplicate. The symbols represent mean ± SEM. The error bars falls inside the symbols.

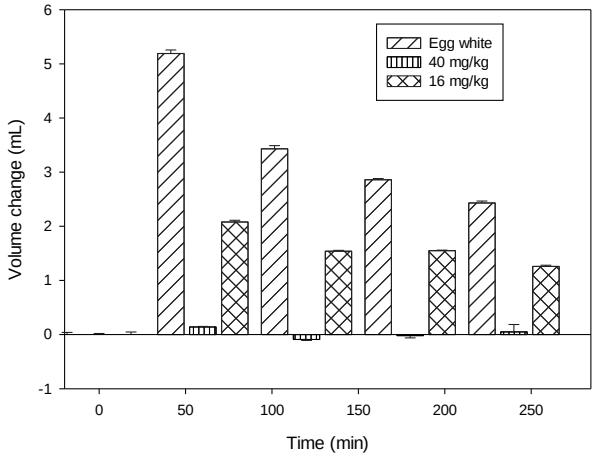

Fig. 3: The figure shows the inflammatory effect of the intraplantar injection of 50 µL of egg white at time zero. It can be observed that the maximum volume change (as a measure of edema) is produced around 60 minutes and lasts at times longer than four hours after injection. Also, the anti-inflammatory effect of ibuprofen i.p. applied at time zero. The figure shows the effect of ibuprofen at two concentrations. At 40 mg/kg, the anti inflammatory effect is complete, and no edema is developed. At lower doses (16 mg/kg) the edema is less prominent and keeps decreasing for all the time of the experiment. The experiments were done in triplicate. The bars correspond to the mean±SEM.

Inflammation or edema formation can be measured by different methods. For example, the *in-vitro* anti-inflammatory study by detecting proteinase inhibitory activity (Kumarappan *et al*., 2006), and the method for cotton-pellet-inducing granuloma (Yang *et al*., 2004), both have the main disadvantage of being time consuming. Measurements of manual dorsoventral paw diameter as a measure of inflammation using a Vernier caliper are not sometimes precise enough to reach reproducible results, especially when dealing with many animals to be under test. Intravenous injection of Evans Blue (Nidavani *et al*., 2014) as a measure of inflammation by testing blood vessel permeability requires killing the animal for making the measurements. This constitutes a problem if you need to perform experiments over time. The devices presented in this work are available in most laboratories, the PowerLab equipment and especially the force transducer. Some advantages of our plethysmograph are the possibility of recording the data obtained from the different experiments by the digitalization provided by the PowerLab equipment. The other plethysmographs lack this feature (excepting the Ugo Basile commercial plethysmograph). Another advantage of this device is the obsolescence of the analog devices with respect to the availability of chart paper or magnetic tape reels. One of the principal applications of this technique is the study of new anti-inflammatory agents, mainly from natural origin (Vinet *et al*., 2014) or medicinal plant extract (Ahmed *et al*., 2014).

Conclusions: In the present study, a plethysmograph for measuring rat paw volume was designed and tested. Results showed that the system is very sensitive and has good reproducibility for making reliable and quantitative measurements of inflammation in the rat paw. Further studies could be oriented to apply this equipment to study the anti inflammatory effects of herbal extracts in laboratory animals.

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Author's contribution: GB performed most of the plethysmograph experiments. CV contributed to diagrams and graphics while JLM made the statistical calculations and wrote part of the manuscript. CL was responsible for the design and testing of the plethysmograph, the analysis of all the experimental data and writing the entire manuscript.

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