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Simple multichannel system for the measurement of the net water flux across biological tissues

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ABSTRACT

This paper describes the development of a simple system for measurement of net water movement through biological membrane barriers. The system is based on the detection of a water meniscus inside a polyethylene tube, which reflects the water movement inside one hemichamber of a modified Ussing chamber containing a membrane barrier. The detection device consists of a commercial computer-controlled flat bed scanner and specifically developed software. This system allows one to perform a relatively high number of individual experiments per physical unit. It is a flexible and affordable device, which allows comparatively more information per unit to be obtained than previously described methods.

1. Introduction

Non-isotopic measurements of net water movement (J_W) across biological barriers were performed initially by means of gravimetric methods. The gravimetric technique described by Bentley [1] has been used in experiments of water transport in different tissues such as urinary bladder, gall bladder and intestine. However, since with this method it is necessary to measure organ weight variations at different times, it cannot be used for short time measurements or under determined experimental conditions, such as rigorous control of hydrostatic pressure. Repetitive tissue handling can possibly also induce damage and variations in the experiments. Modern computerized gravimetric approaches have been recently used to quantify osmosis and filtration in intact mouse lung [2].

Bourguet and Jard were the first to use another experimental approach, the volumetric technique, to measure water movements across epithelia [3]. Their method involved a mechanized device that automatically replaced water loss in the mucosal side of a modified Ussing chamber where a tissue was placed. The volume of water replaced represented the volume of fluid crossing the tissue. This device was responsive and sensitive but it had multiple mechanical parts and a complex calibration mechanism. Moreover, it was not able to respond automatically to changes from secretor to absorptive states or vice versa, since it needed manual adjustment for each condition.

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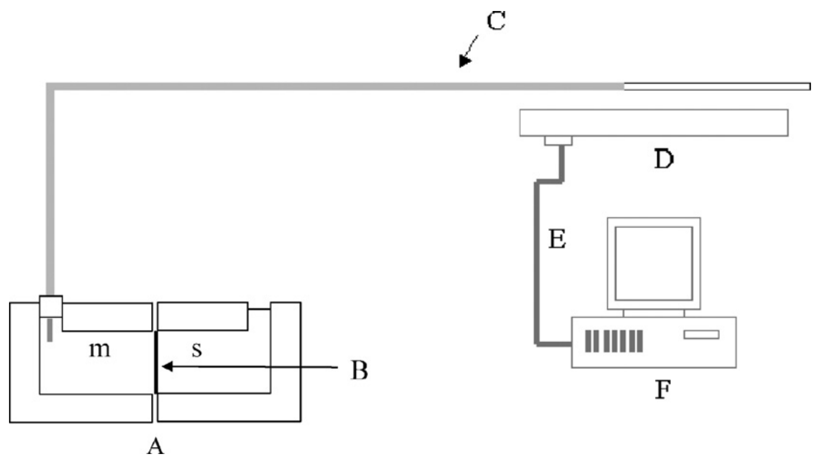

Fig. 1 – Diagram of the multichannel system used to measure and to register net water fluxes across biological membranes. Complete system: (A) thermostated chamber with the tissue; (B) tissue (m: mucosal side; s: serosal side); (C) plastic tube with saline solution; (D) scanner-based detection system; (E) connection between the scanner and the computer; (F) computer. The gas port to bubble the tissue with CO_2–O_2 is not shown. Figures are out of scale.

Dorr et al. later developed a computer-based data-acquisition system for the automatic measurement of net water fluxes based on the volumetric technique [4]. This device allowed accurate and continuous determination of net water movement across any biological barrier, detecting changes of net water flux from secretory to absorptive states in real time. The technique is based on the detection of a liquid meniscus inside a capillary tube using an electro-optical device connected to a computer. The method was successfully used in different experiments of water transport, both in animal [5–8] and plant tissues [9]. However, this design requires complex connections such as an intermediate chamber between the tissue chamber and the optical device, and it was designed as a single-channel instrument, i.e. a complete device is required to perform each individual experiment. In addition, this equipment is relatively expensive. These limitations restrict the use of this system.

Over recent years, significant progress has been made in the development of digital systems for the processing and handling of large amounts of data at increasing speeds. At the same time, ordinary image capture devices such as desktop scanners have become more sophisticated and inexpensive. Taking into account these technical developments and the advantages of a computerized volumetric technique, it has been possible to design a new improved system for the measurement of net water fluxes across tissues using these affordable optical devices.

The purpose of this work is to introduce a simple, affordable and practical multichannel system that allows simultaneous measurements of the net water movement across several biological barriers by using a commercial scanner.

2. Materials and methods

2.1. Multichannel optical system detection

The system presented here was based on the digital detection of a meniscus into a polyethylene tube resting on a reflexive light bed scanner controlled by a computer. The meniscus represented an air–solution interface and its displacement reflected the water movement across a tissue mounted as a diaphragm in an Ussing chamber. This was possible because of connections between the polyethylene tube and the mucosal side of the chamber (Fig. 1), which was hermetically sealed with a silicon stopper. A stainless steel needle inserted through the stopper connected the polyethylene tube with the mucosal side of the chamber, which contained saline solution. The polyethylene tube was placed on the scanner in order to detect the position of the saline solution meniscus inside the tube. In the multichannel set-up, a set of polyethylene tubes, each of them connected to a different Ussing chamber, was supported on the same scanner bed.

2.2. System description

2.2.1. Hardware

A commercial Canonscan Lide 20 scanner (Canon, USA) with an optical scanning resolution of 600×1200 dpi, color 48 bit internal/24 bit external and maximum scanning area 8.5 in. × 11.7 in., was used to develop the new set-up. The scanner was controlled by means of a personal computer running the Windows operating system (Microsoft, USA) with a minimum configuration based on a 700 MHz Pentium III microprocessor, 128 kbytes of RAM and 2 Gbytes hard disk.

The polyethylene tubes rested on the scanner bed by means of a specially designed support that assured stability and positioning (Fig. 2a and b).

2.2.2. Software

A specially developed program drove the image capturing system using a standard software protocol (Twainwizard, Mediarch GmbH, Germany) and the application interface.

The software controlled the displacement of the optical and acquisition systems of the flatbed scanner and the size of the area to be scanned. The image obtained was stored