Aqueous humor fluid dynamics can be better understood by a real time perfusion system at constant pressure

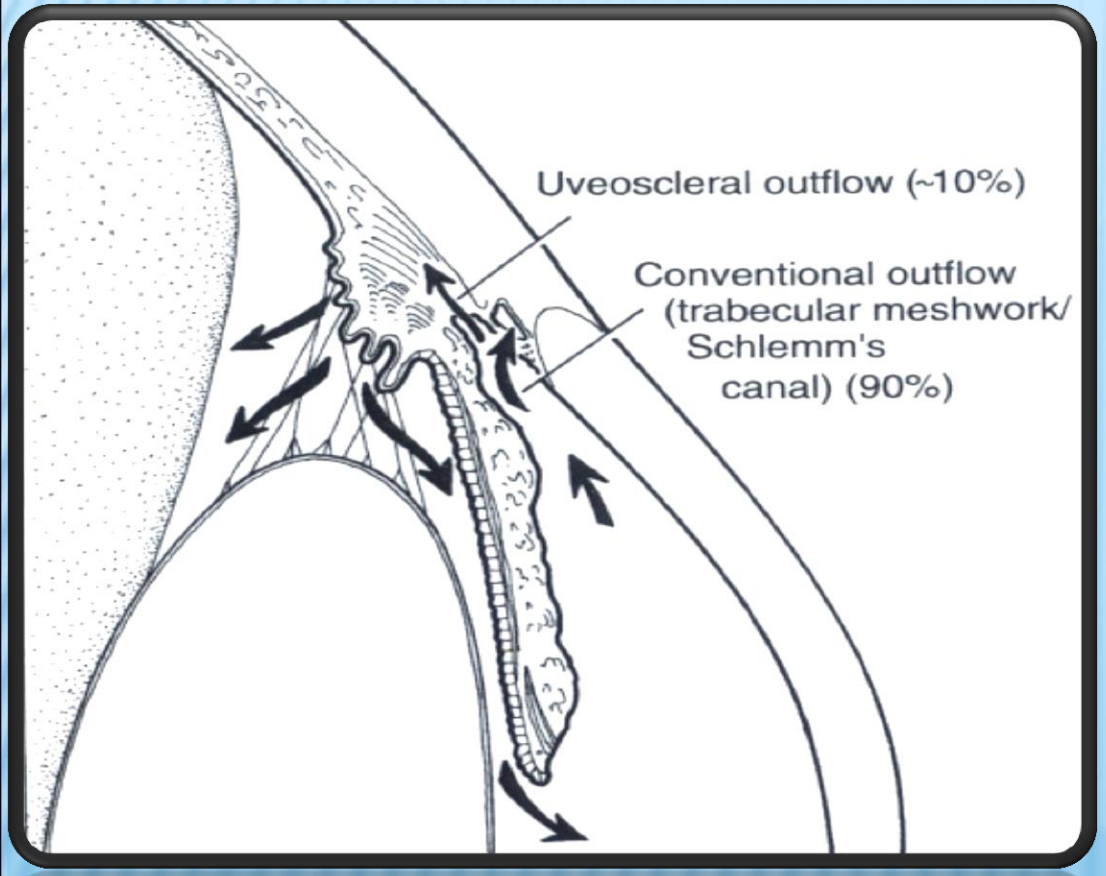
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Glaucoma is a progressive neuropathy;

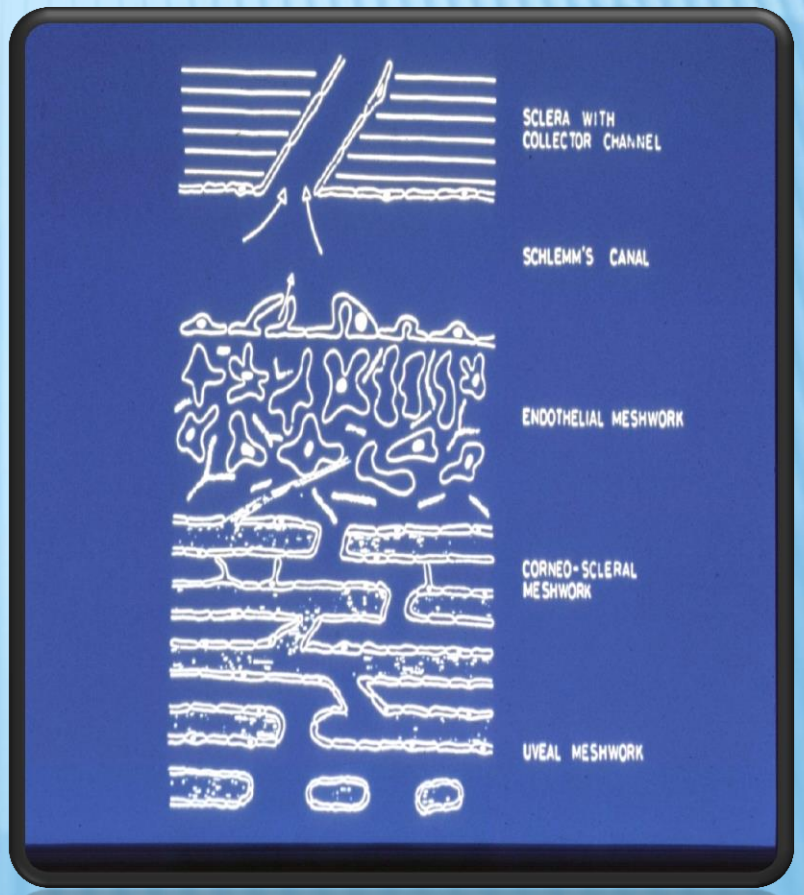
- Generally, associated with elevated IOP
- But, the mechanism of aqueous humor fluid dynamics in normal eye or aqueous humor outflow resistance in glaucomatous eye remains unknown.

Causing hindrance for the development Of effective glaucoma therapy.

Schematic presentation of aqueous humor fluid dynamics in anterior chamber of the human eye



Direction of fluid flow



Conventional outflow

For quantitative determination of the drug's effect on the aqueous outflow facility and/or the regulation of aqueous outflow resistance to the conventional outflow pathway, the following techniques are widely used in the field of glaucoma research

a. Grant perfusion system
(at constant pressure)
b. Organ culture perfusion system
(at constant inflow)

Goldmann equation for pressure measurement

$$P=F/C+Pe$$

P- Pressure (IOP)
F-Flow µl/min
C-Outflow facility µl/min/mmHg
Pe-episcleral venous pressure
(8 mm Hg assuming constant)

Based on the pharmacological/histological analysis, the regulation of outflow facility predicted the role of

A. cell-cell interactions in the outflow pathway cells.

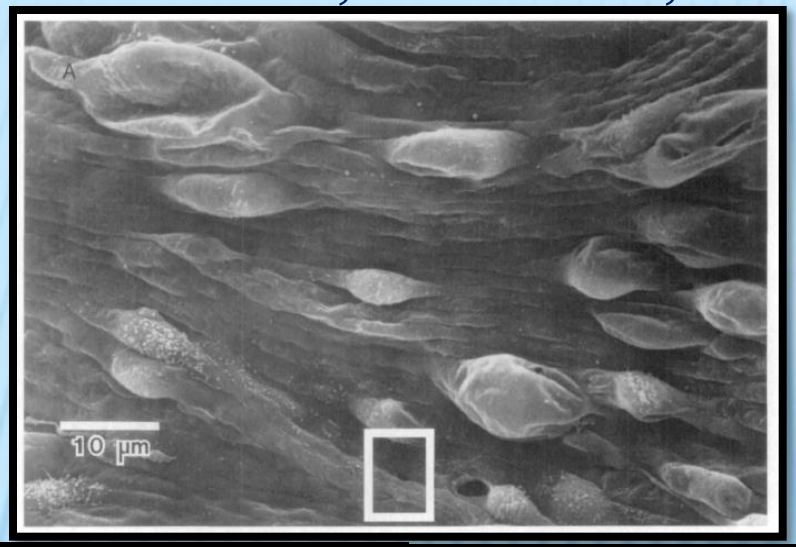
B. Two types of pores (intracellular and intercellular) in the inner-wall of Schlemm's canal.

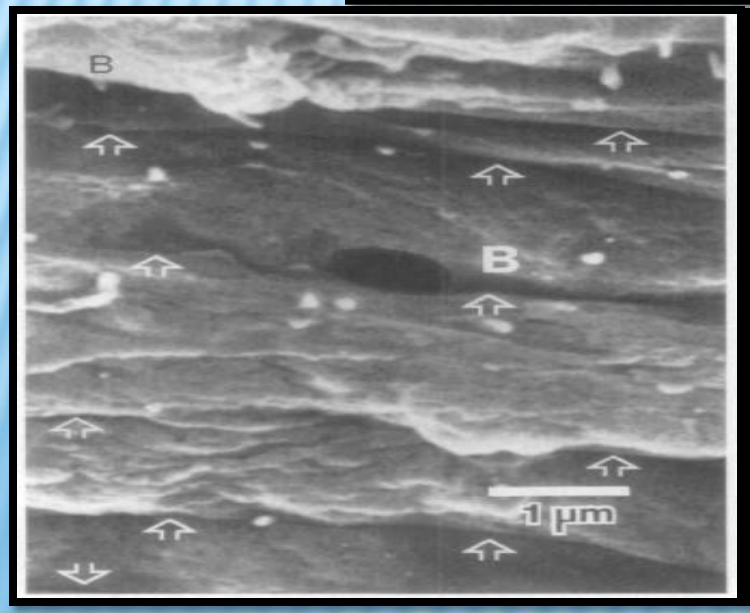
CR Ethier, FM Coloma, AJ Sit and Mark Johnson IOVS 1998; 39: 2041-2048.

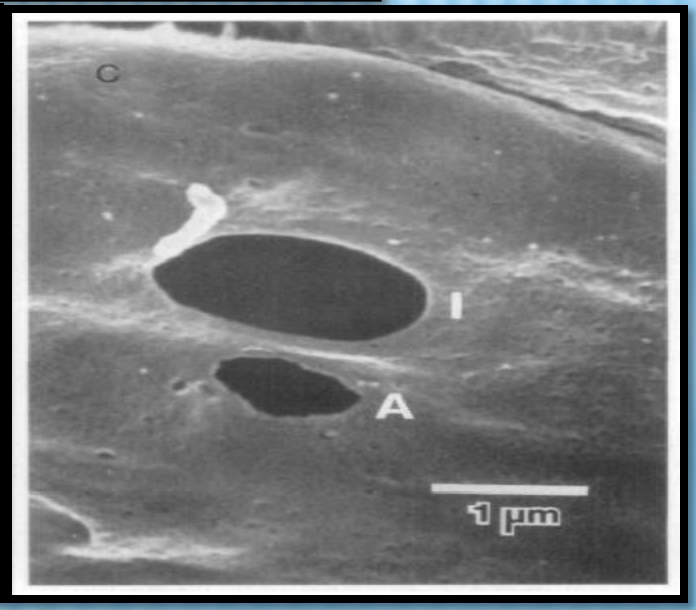
(unfortunately not yet accepted widely)

Scanning electron photomicrographs of pores In inner-wall of SC endothelium

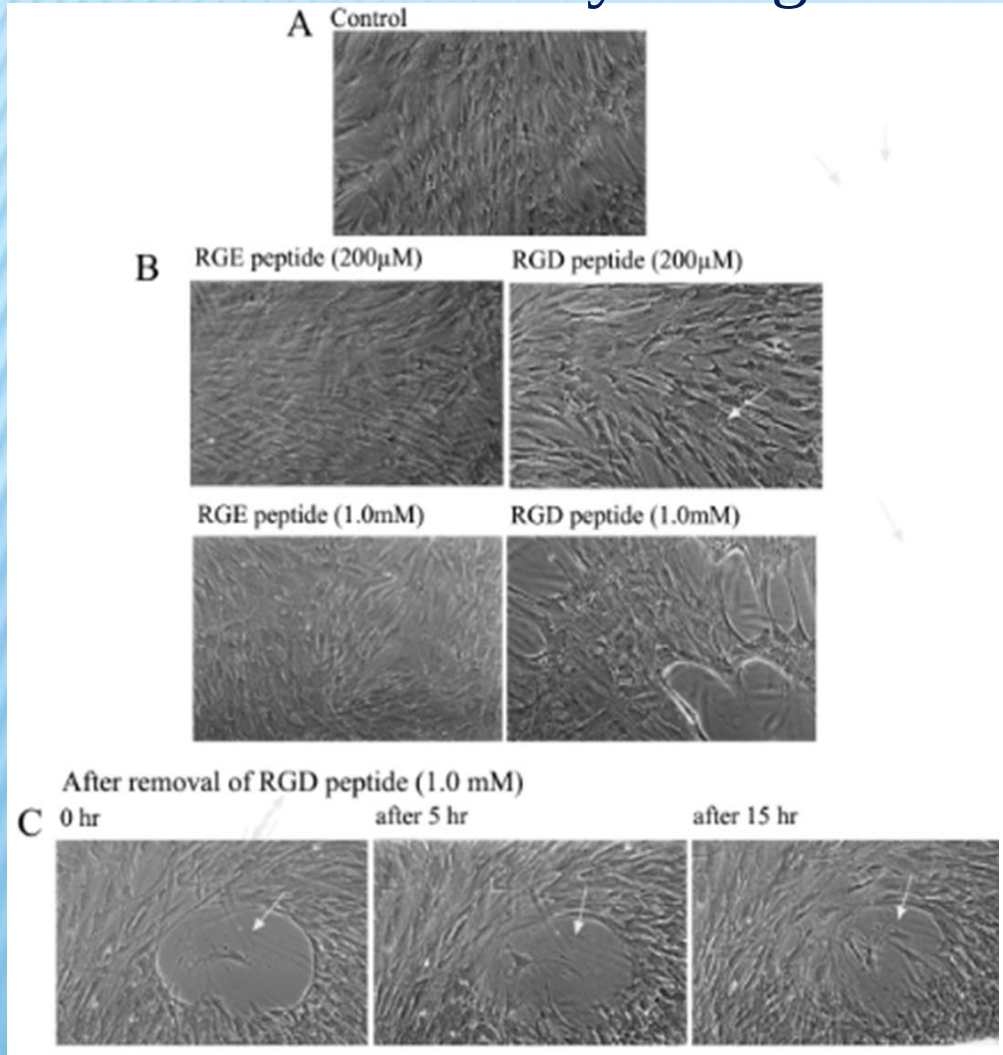
Either CR et. Al., IOVS 1998; 39: 2041

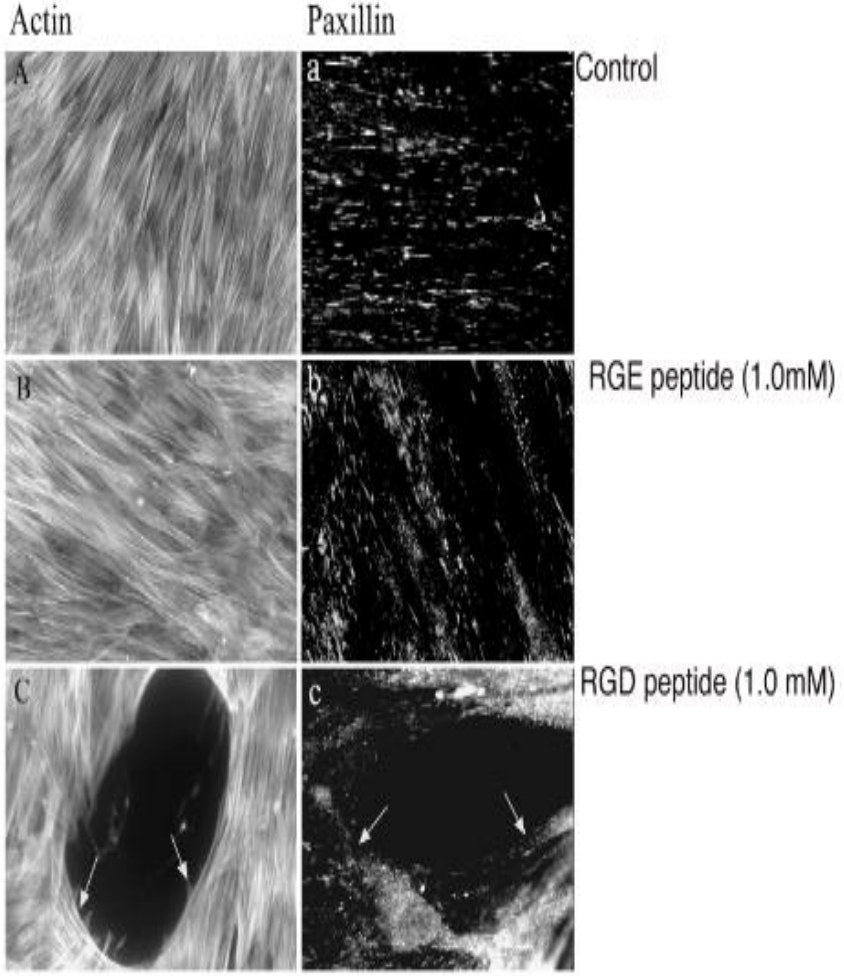






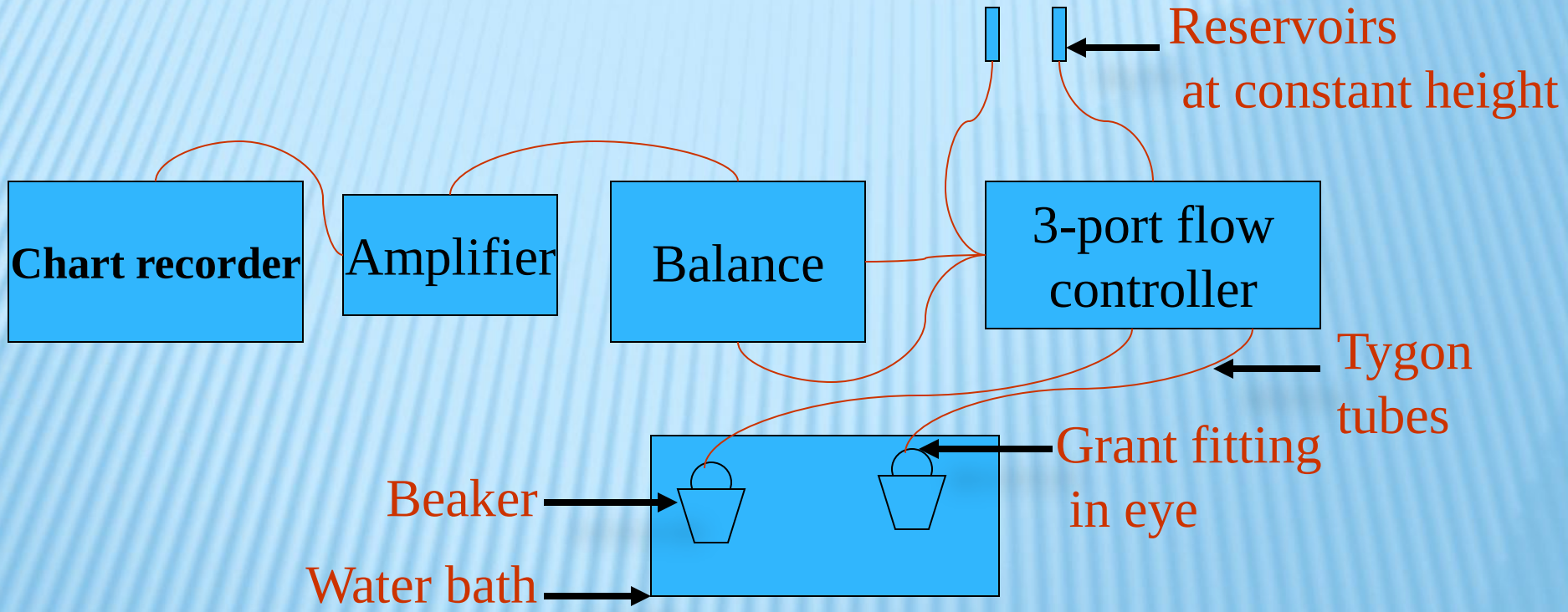
In vitro studies of RGDTP peptide effects on SC monolayer (unpublished data) supported the two pore hypothesis but contradicted by Doug Johnson IOVS 2004;45: 2246.





Major problem for the studies of aqueous humor dynamics in the eyes that the perfusion systems are self engineered and not available commercially, worldwide. Therefore, understanding of the perfusion systems are needed.

Schematic representation of Grant perfusion system to measure outflow facility in eye



Overview:

Grant perfusion system developed by W.Morton Grant (1955) at MEEI, Harvard University Medical School, Boston. Images taken after permission of Dr. Epstein, DUMC, NC



Components of Grant perfusion system:



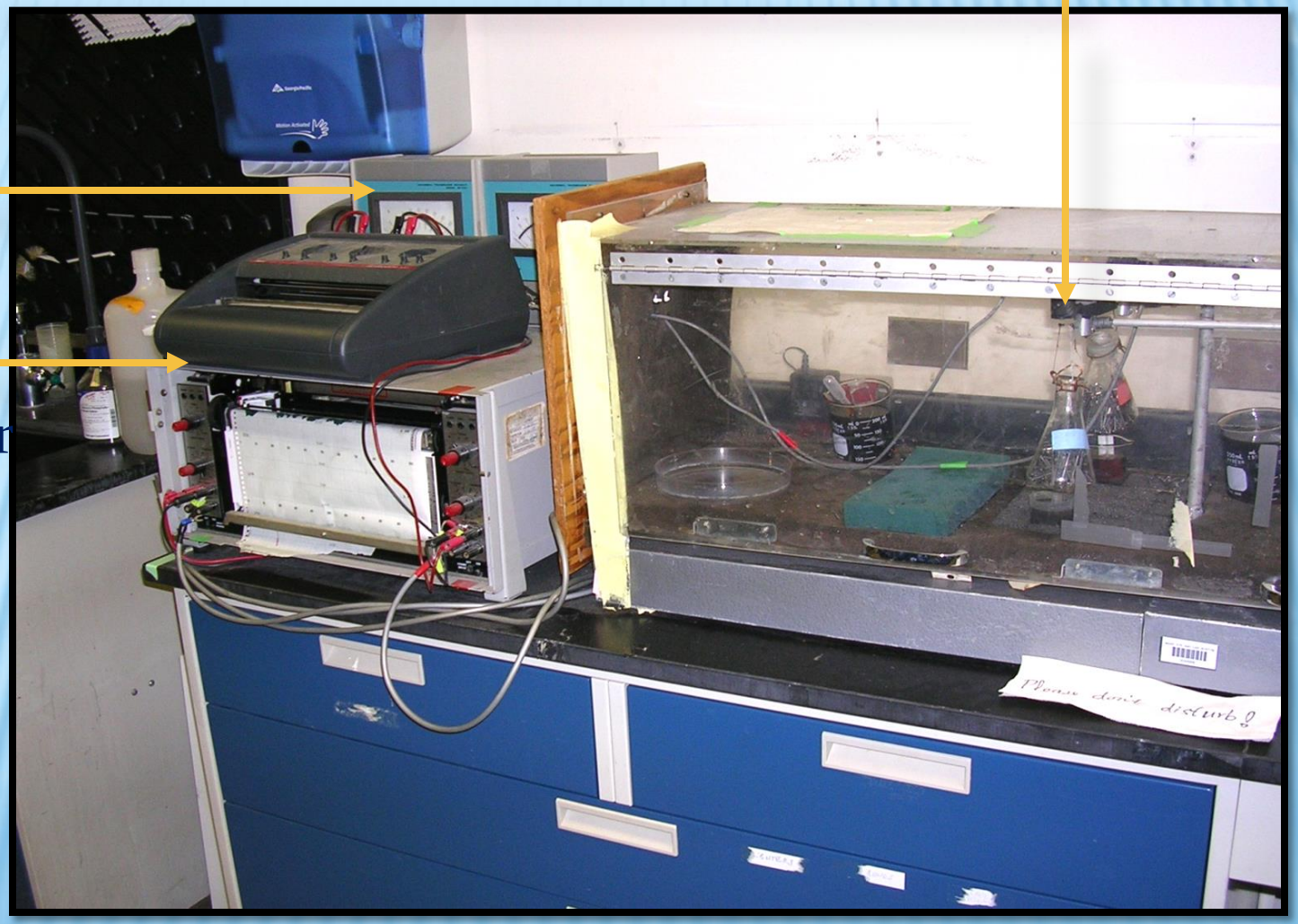
Hanging cups to measure fluid loss

Fluid Reservoirs at a fixed height

Fluid flow 3-port controllers

Water bath to place eyes after stainless steel Grant fitting connected with the tube of flow system

Components of Grant perfusion system



Magnified view of the balance system

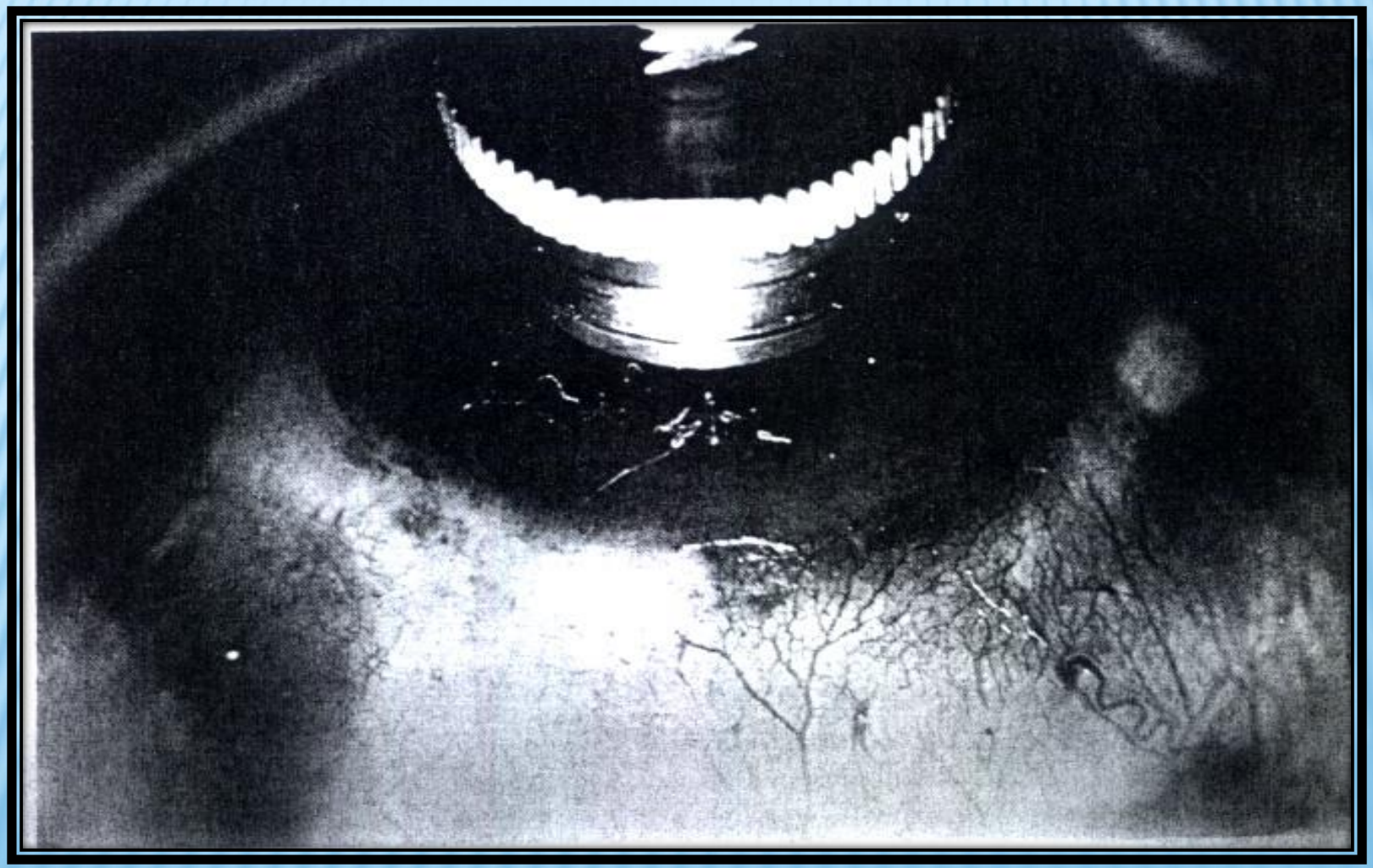


A view for the arrangement of the eyes placement on moist paper cones support containing beakers in the water bath temperature maintained at 25°C after stainless steel Grant fittings

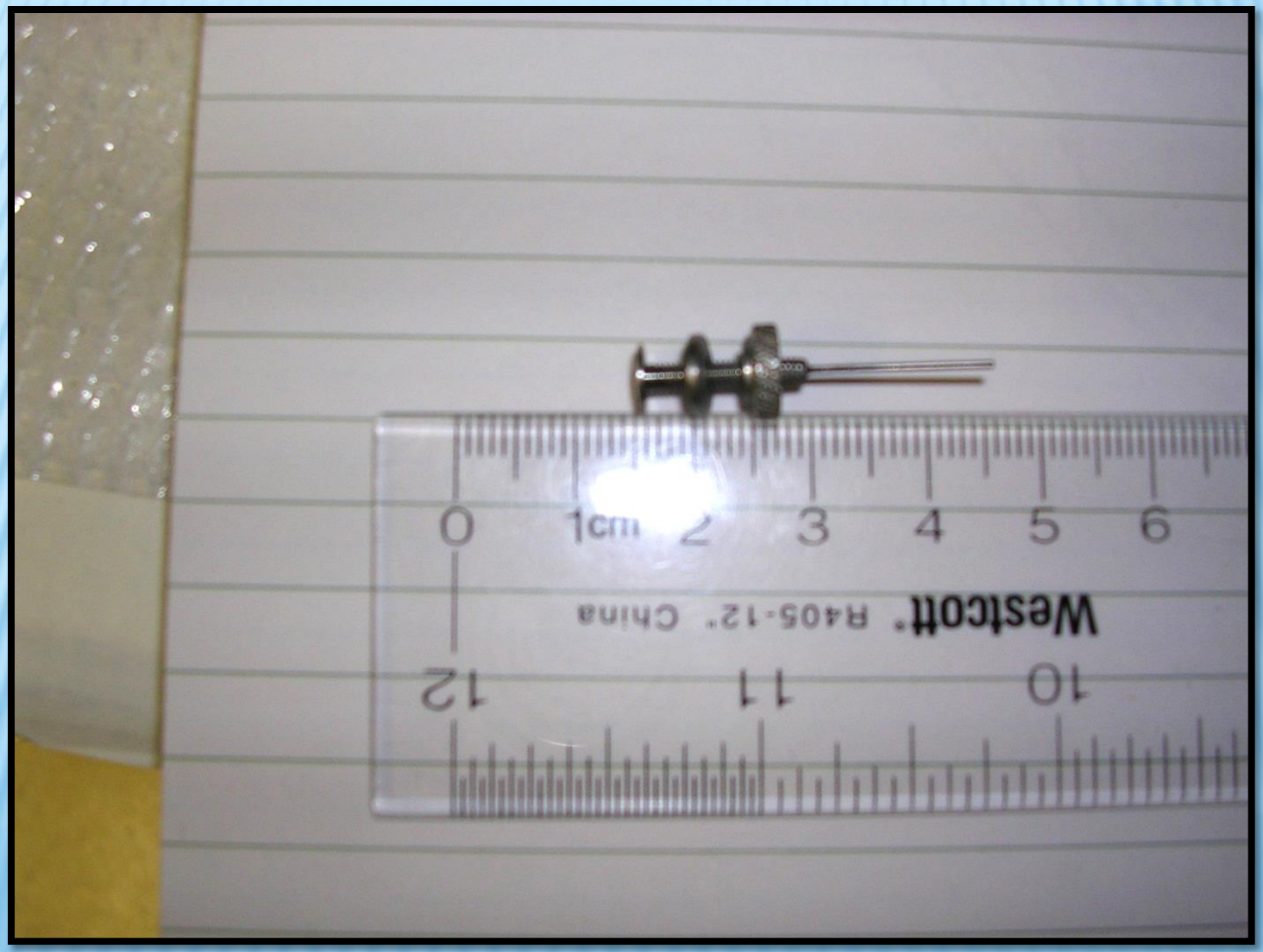


The tubes connect to the anterior chamber of the eye through the Grant fittings.

The stainless steel Grant fitting in a hole trephined in the cornea



An overview of the components of the Grant fittings.



Grant perfusion system

Merits:

a. The provision (Grant fitting) is made for tight reclosure of the opening in the cornea, and connecting to the tube for determining the facility of intraocular fluid by the perfusion method of the whole enucleated eyes. The results are readily available and used within twelve hours after procurement.

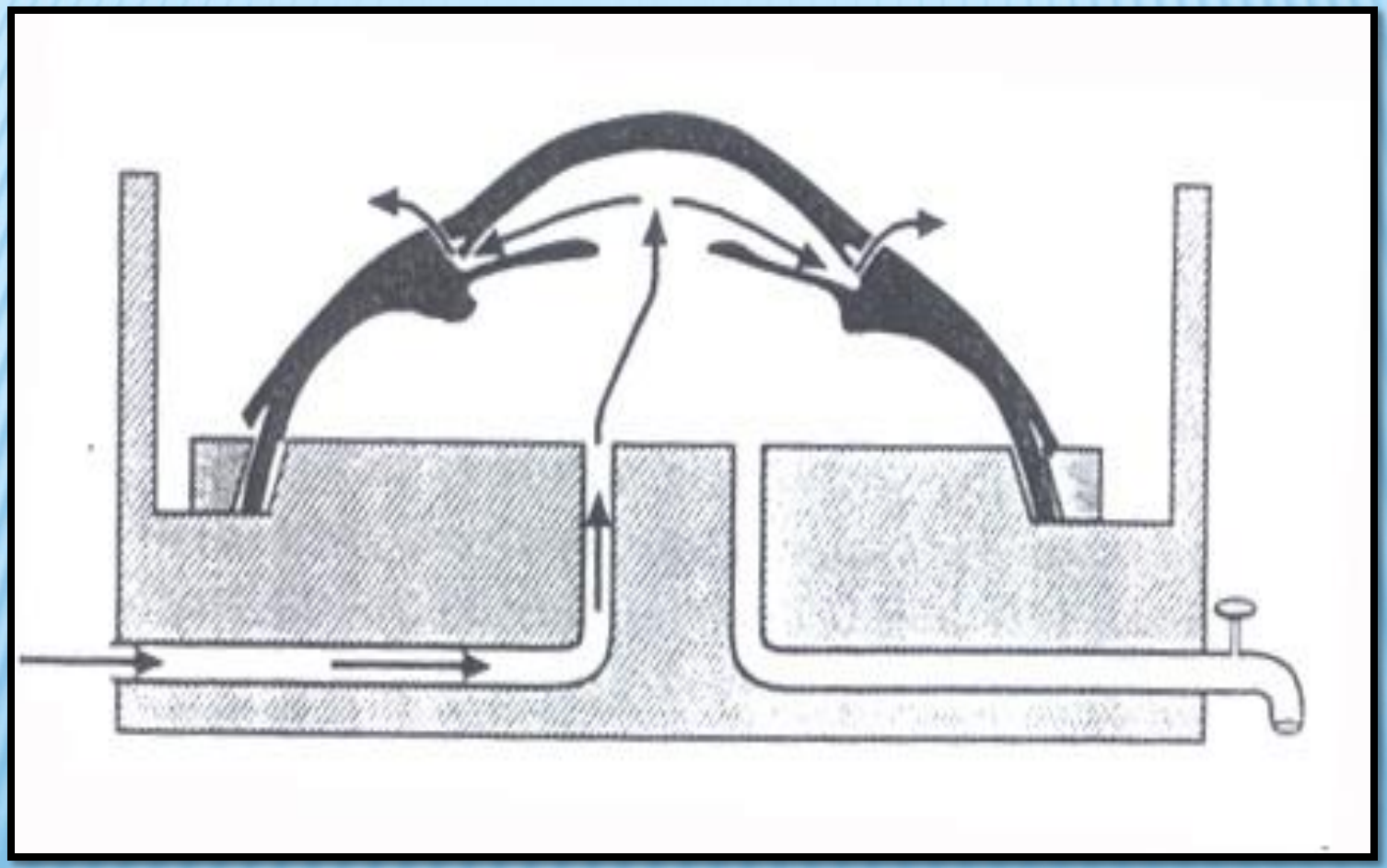
b. The anterior chamber can be cleaned and rinsed with drugs that is important for immediate exposure to outflow pathway cells.

c. Iridectomy can be performed so that will not interfere with the angle closure.

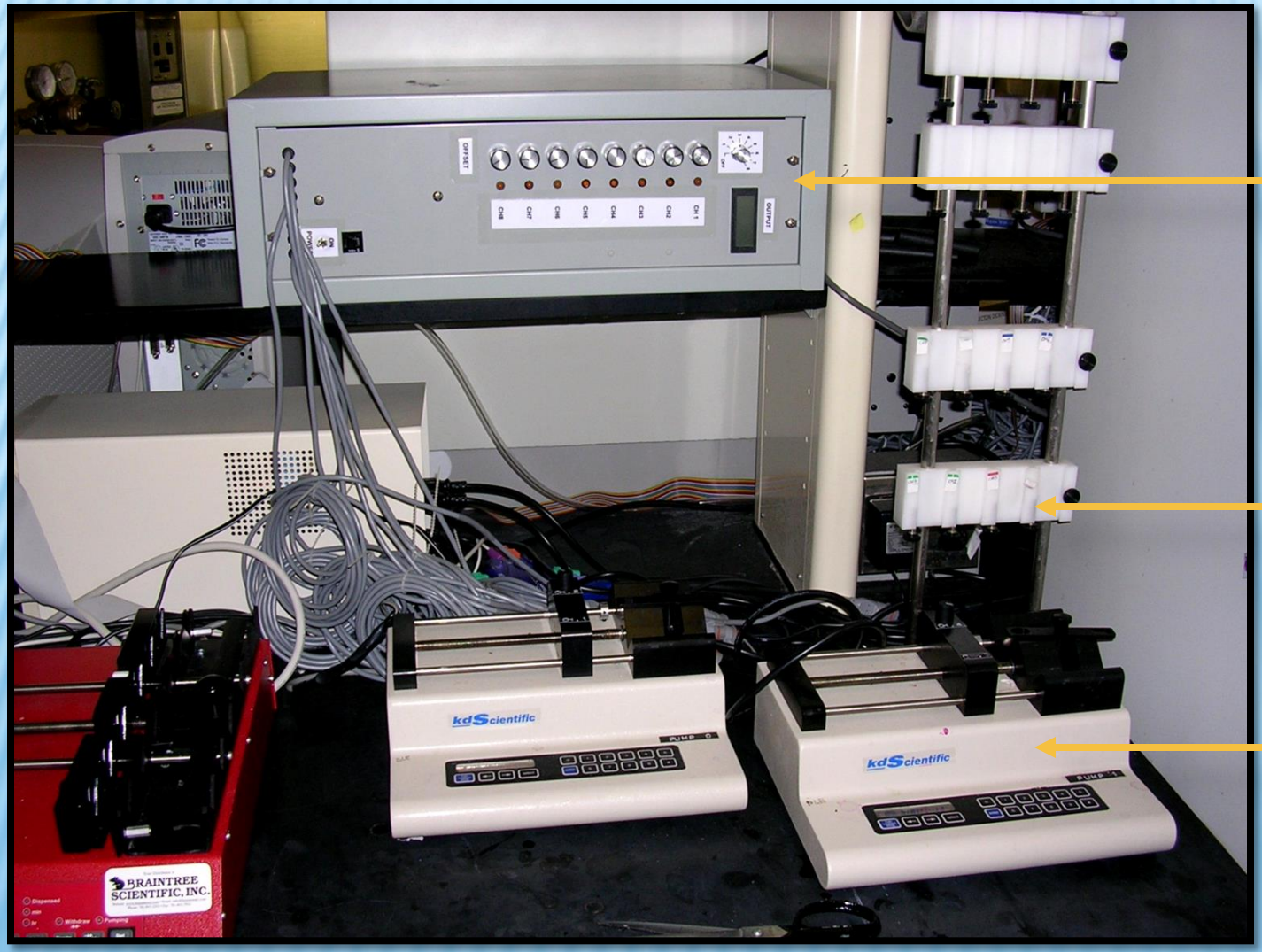
Demerits:

a. Very old system of the balance with hanging buckets so that the measurements can be influenced by the vibrations and/or other disturbances.

Schematic model for the cross section of organ culture to show the flow of medium through eye



An overview of Organ culture perfusion system at constant flow:



8-channels Amplifier, made by Biophysics dept at Duke.

Stand to hold pressure transducer

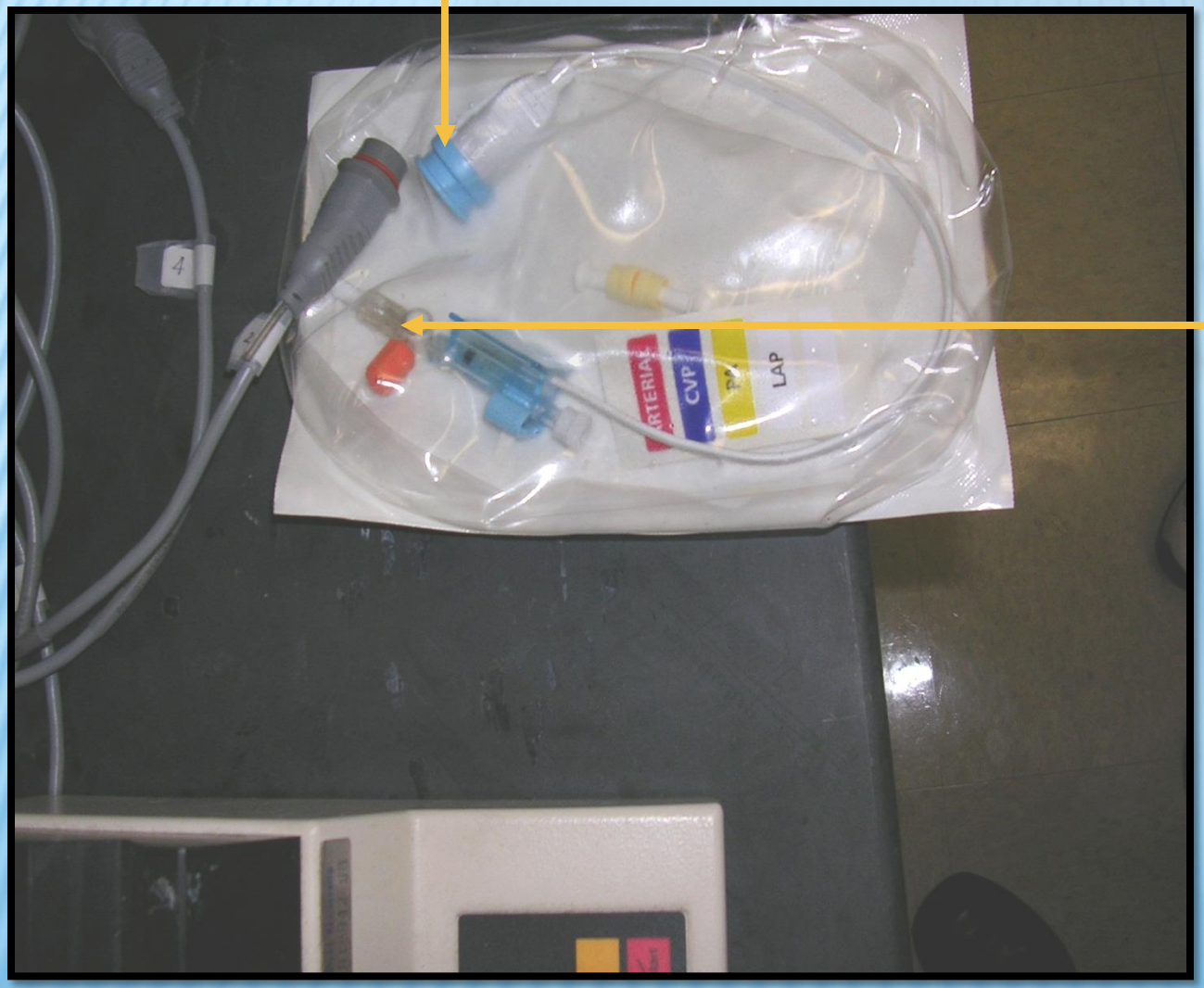
Constant flow pump (Harvard instrument)

Components of Organ culture perfusion system



Organ Culture dish placed in CO_2 incubator ; inflow cannula connects to constant flow pump, and out flow cannula connects to the pressure transducer

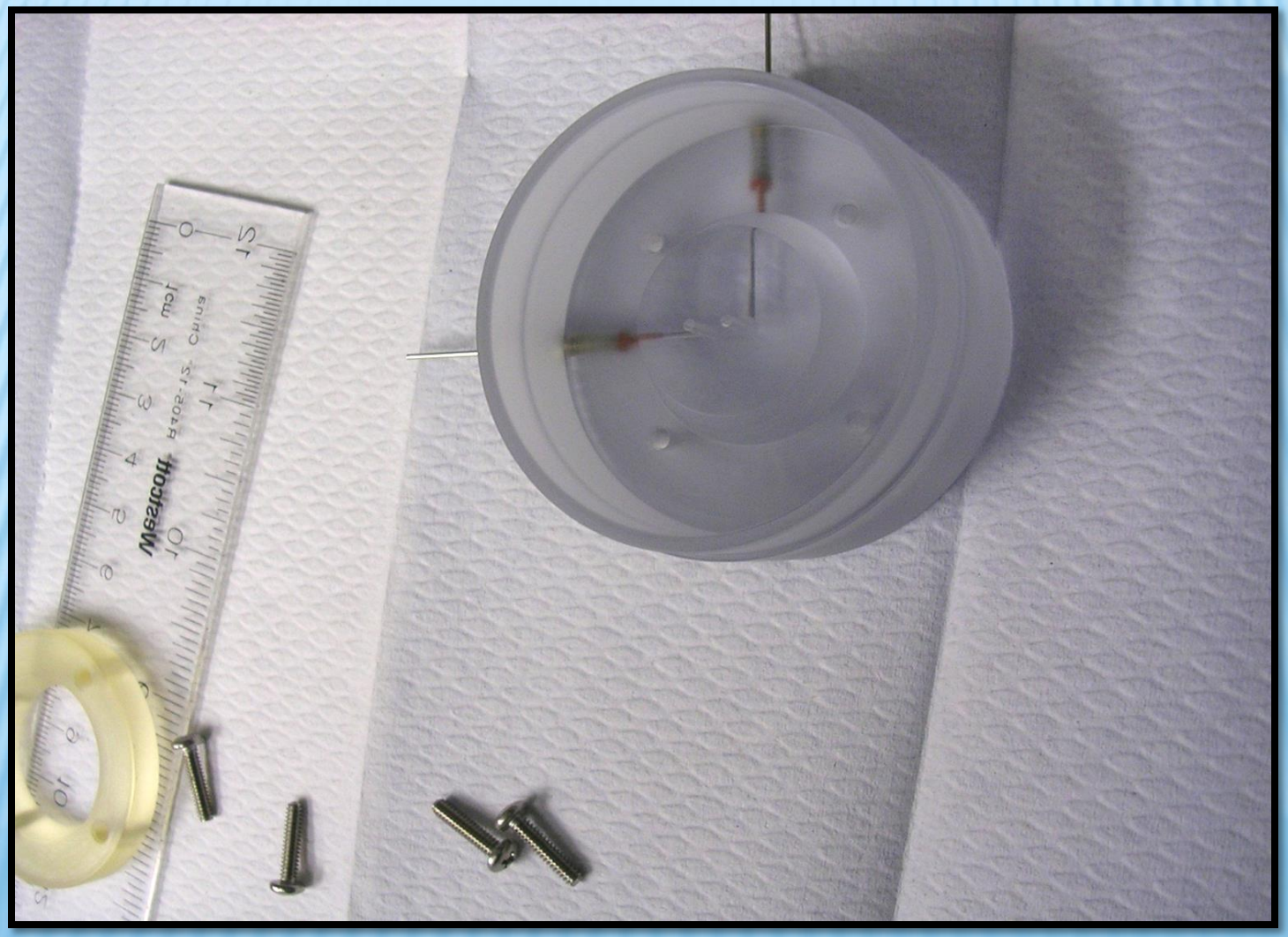
Magnified view of the pressure transducer for Organ culture system:



One end of pressure transducer connects to the amplifier and signals exported to the computer.

Outflow cannula of the organ culture dish connects to the pressure transducer

Top side view of the organ culture dish to fit the O-ring



Organ culture system:

Merits:

a. This system consists of a controlled fluid flow and perfused with culture medium.

b. IOP can be monitored upto 6 pairs of eyes at a time for three to four weeks without cell death.

Demerits:

a. Half a globe of the eye is used in this method whereas iris, cilliary bodies, lens, vitreous are removed.

b. Culture medium is perfused without serum that might cause depolymerization of cytoskeleton, and ultimately to cellular dynamics of the outflow pathway cells.

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A new approach to develop a real time perfusion system.

designed by Janardan Kumar at Tufts University, Boston and presented at ARVO (2006).

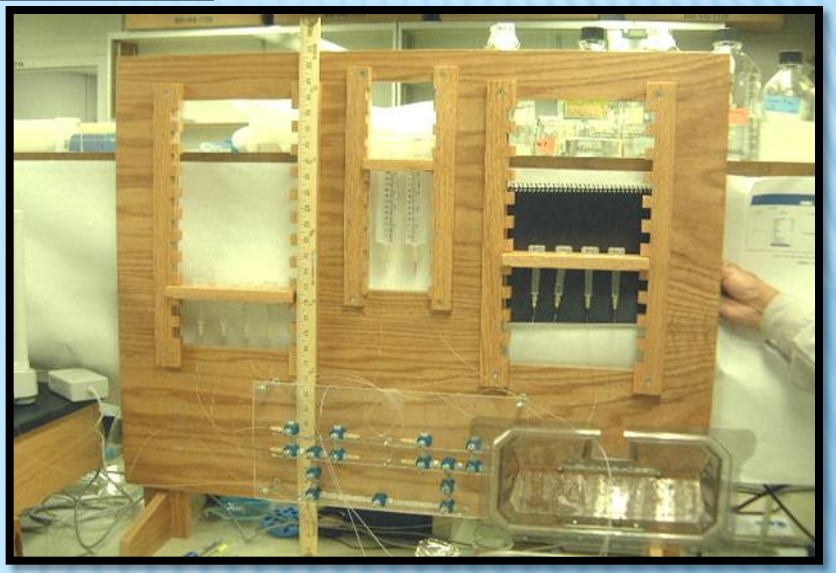


An overview of the real time perfusion system and its major components

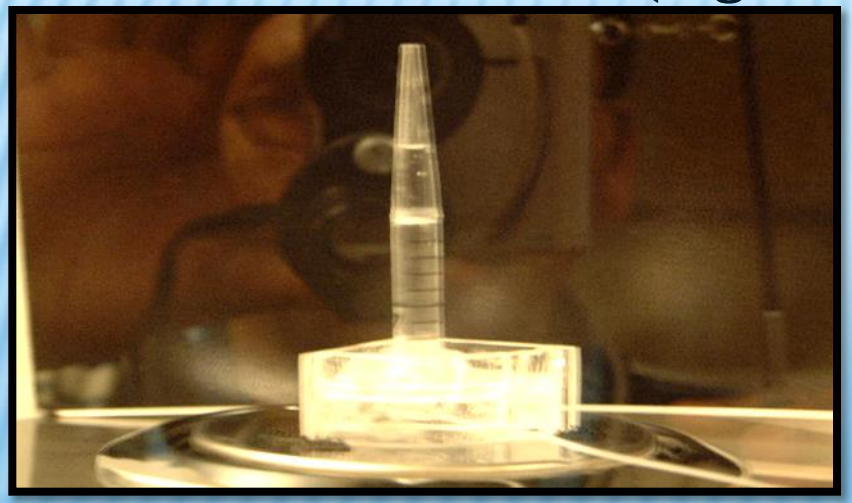
Special features of the system:



A. Balance Mettler Toledo (digital)



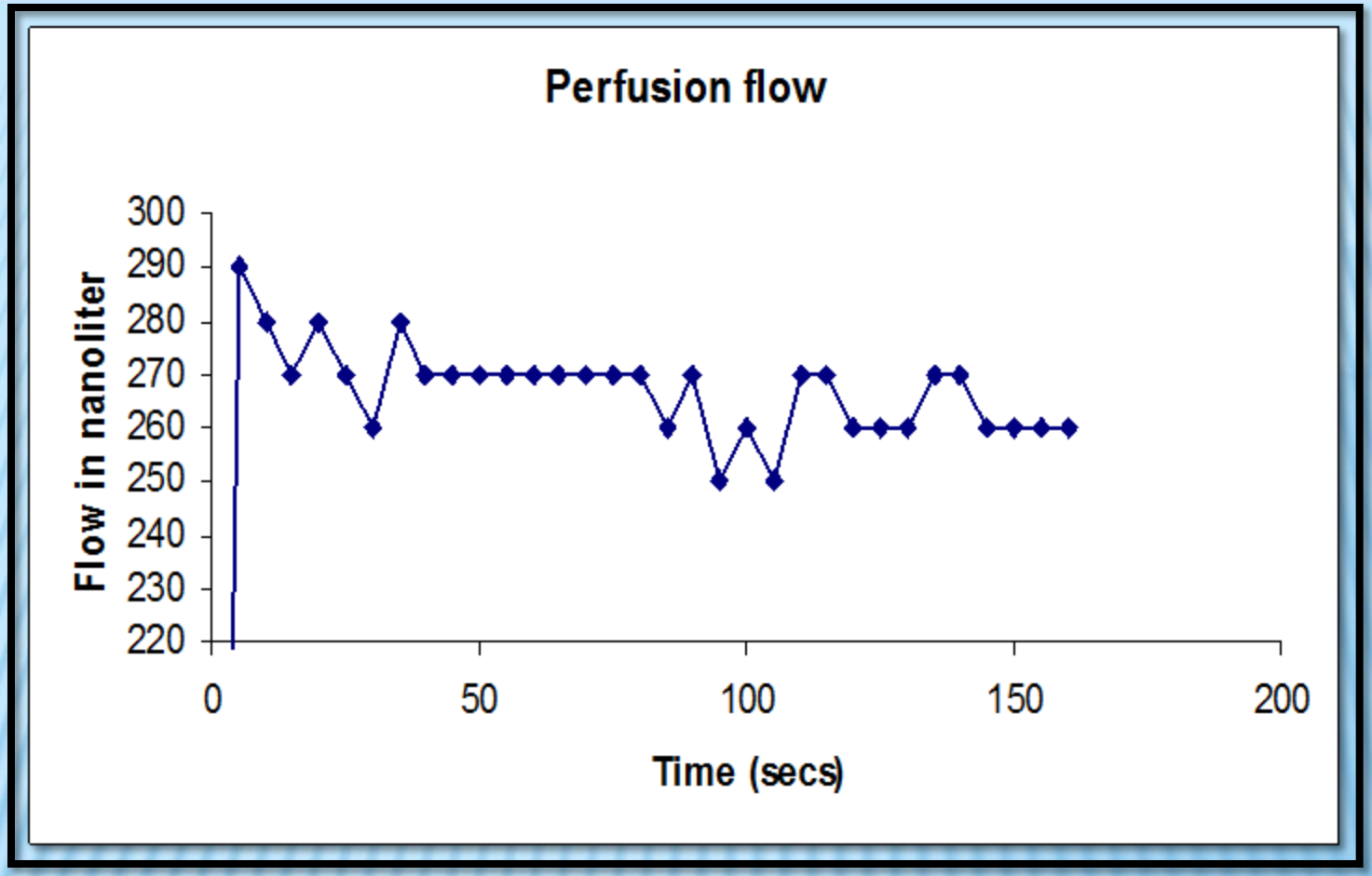
C. Device to maintain constant pressure vs. height of reservoir



B. Fixed reservoir on the balance to reduce evaporation and vibration



D. Real time measurement by Balance tallk XL program



A preliminary data of outflow in a whole porcine eye obtained by using the real time perfusion system with needle cannulation, indicating that aqueous humor outflow is discontinuous. Unique feature of this device is that the outflow can be measured as per second and easy to develop the device in any laboratory worldwide.

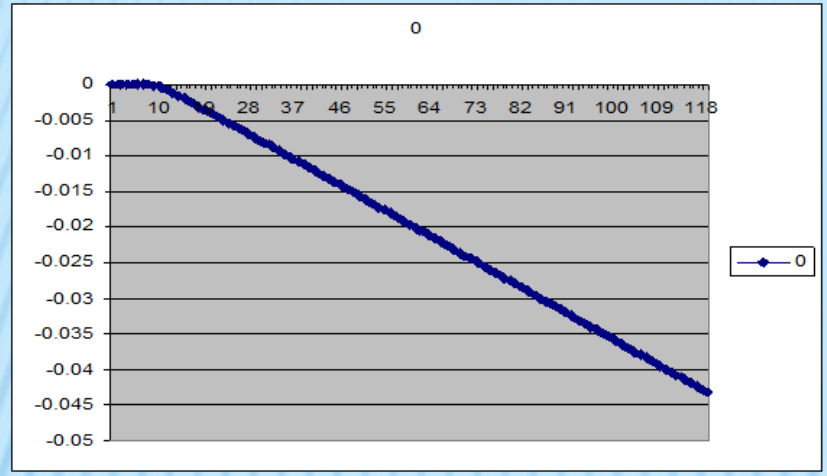
Comparative analysis of perfusion medium for the baseline outflow facility measurements using real time perfusion system

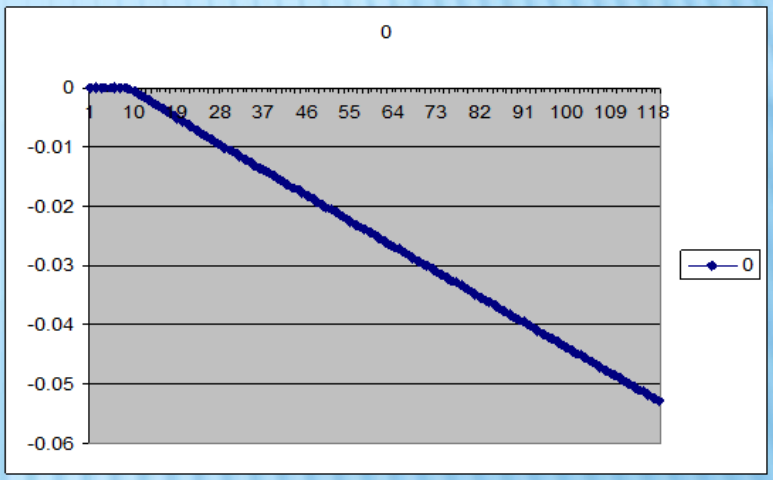
Perfusion medium

PBS + Glucose

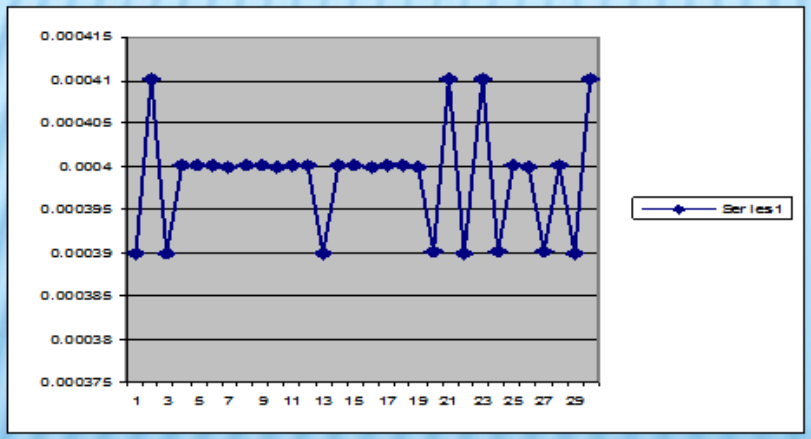
Opti-MEM

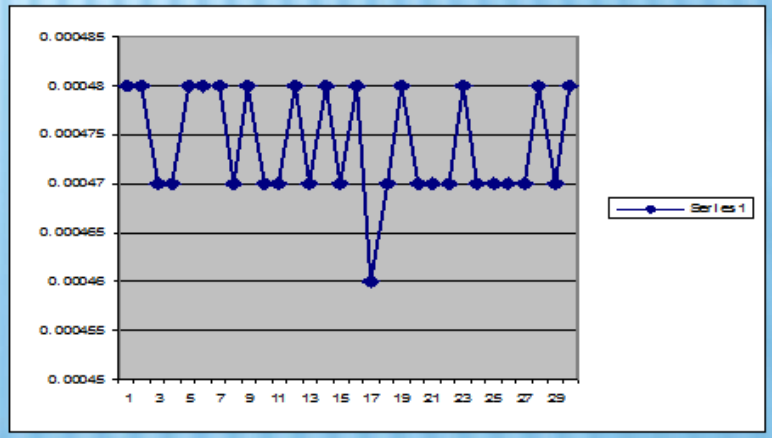
Real time baseline graph as weight loss





Expansion of outflow data point/sec





Average=0.000399 gm/5sec
=0.004788 gm/min
=0.000319 gm/min/mmHg
Outflow facility=0.32 µl/min/mmHg

Average=0.000474 gm/5 sec
=0.005688 gm/ min
= 0.000379 gm/min/mmHg
Outflow facility= 0.38µl/min/mmHg

Conclusions:

A. The real time perfusion system that measures the outflow facility at constant pressure, will resolve the mystery that the aqueous humor outflow in the eye in ex-vivo is either continuous or discontinuous.

B. If the outflow is discontinuous then the two pores hypothesis in the inner wall of SC led by Mark Johnson would be strongly supported.

C. Aqueous humor outflow can be measured at any given time points in seconds.

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2. Prof. J Duker, and
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