A microanalysis of colloid osmotic pressure[1]

Y. Kakiuchi, G.M. Hughes[2], T. Arai, M. Horimoto, Y. Kikuchi and T. Koyama

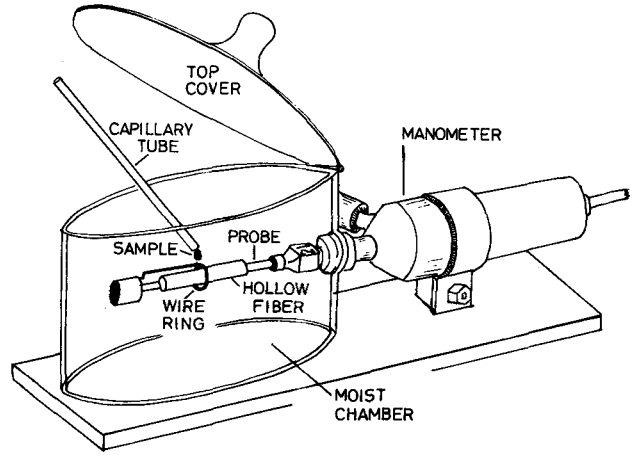
Division of Physiology, Research Institute of Applied Electricity, Hokkaido University, Sapporo, Hokkaido 060 (Japan), 11 March 1980

Summary. A needle type of colloid osmometer has been devised with special attachments which made it useable with 3-µl samples. It has been used in oncometry of fresh water teleosts to give the following results: 5.1 ± 1.0 mm Hg for goldfish, 5.2 ± 0.71 mm Hg for carp, and 14.3 ± 0.75 mm Hg for eel.

A miniaturized and well-organized membrane osmometer originating from Hansen[3] has been developed to measure colloid osmotic pressure (COP) of µl samples by Wiederhielm et al.[6], however its intricate construction made it difficult for us to follow their method. To facilitate osmometry of small amounts of samples such as blood, lymph, interstitial fluid, or edema fluid, improvements have been made using a needle-type colloid osmometer[4]. This paper describes the construction and performance of the osmometer with some results of its application to COP measurement of the blood of fishes.

Methods. A needle type colloid osmometer (detailed description in a previous paper[4]) is used with an auxiliary adaptor suitable for use with small sample volumes. The construction is illustrated in the figure, in which the osmometer is mounted horizontally for convenience of both rinsing and sample loading. A moist chamber containing a suitable amount (5 ml) of water at the bottom is made of Lucite (50 mm inner diameter, 50 mm height with a hinged top cover), and contains the osmometer in order to keep the probe from drying. The probe is a COP sensitive unit composed of: a) a 21-gauge (0.8 mm outer diameter) hypodermic needle with 2 perforations drilled through its wall, and b) a 10-mm piece of a hollow fibre (C5P, retentivity: 13,000 mol. wt, Asahi Chemical Co.) threaded over the needle served as a semipermeable membrane to cover its side holes. A wire ring (0.3-mm platinum wire circle of 2.5 mm diameter) surrounding the probe over the side holes plays an important role in trapping and retaining a fluid sample around the hollow fibre surface across which osmosis occurs. A fluid sample is introduced to the ring from either a syringe or a capillary tube. To prevent any contamination or dilution of the sample during measurement, special caution is needed to remove the rinsing water trapped at the ring with blotting paper just before loading the sample. At least 3 µl of sample fluid is required to achieve a reliable measurement, and 2 min is needed for completion of the response. Following each measurement the probe is rinsed by flushing with isotonic physiological saline, and maintained wet. In the fish experiment, 12 goldfishes *(Carassius carassius)* weighing 18–50 g, 3 carps *(Cyprinus carpio)* weighing 450–750 g, and 8 eels *(Anguilla japonica)* weighing 150–270 g were used. Prior to blood sampling the gills were wiped off carefully, then a small incision was made to bleed at an appropriate part of the gills. (In the case of eels, exposure of gills was preceded by incision of the opercular opening). Heparinized glass capillary tubes (microhematocrit tube of 50 µl sample capacity, 1.2 mm inner diameter × 75 mm length, Drummond Scientific Co.) were used to collect blood. About 20 µl of blood, i.e., 10 µl each in 2 separate tubes for duplicate COP measurement, was collected on each occasion. The blood sample was loaded directly from the capillary tube on to the probe by using a microsample injector (Radiometer). Water temperature ranged from 18 °C to 22 °C.

Results and discussion. The performance of the osmometer with a minute quantity of sample was tested by reducing the volume of the test solutions (either 2.5% or 5.0% albumin solutions) from 20 µl–0.2 µl with a micropipette (Pipetman P-20, Gilson). The osmometer responded satisfactorily along the pressure-concentration curve of Landis and Pappenheimer[5] down to a test fluid volume of 3 µl or more, however it often misread when the test fluid was 1 µl or less. Blood COP data are tabulated in the table, in which each pair of measured values is averaged first, then a statistical process followed. Values given are the control value and that obtained 6 h later, and are expressed in the form of mean ± SD. Values for both goldfish and carp are similar but much lower than those for eel. A remarkable decrease (− 10%) in COP appears only in the data at 6 h for the goldfish. It is probably due to excessive bleeding from the incision at the gills for blood sampling, which necessarily results in hemodilution to compensate for loss of blood.



The colloid osmometer system. The system is based on a needle type colloid osmometer of simple structure. The moist chamber and the wire ring are the major accessories to facilitate oncometry of a 3-µl sample, which is loaded from a capillary tube to the probe. About 5 ml of water is deposited in the moist chamber to evaporate. A pressure transducer (P231D, Statham) is used as the manometer. Sufficient stability is obtainable when the system is mounted in a temperature-controlled water bath.

Comparison of blood COP among goldfish, carp, and eel

	Goldfish		Carp		Eel	
	n	COP (mm Hg)	n	COP (mm Hg)	n	COP (mm Hg)
Control	12	5.1 ± 1.0	3	5.2 ± 0.71	8	14.3 ± 0.75
6 h later	10	4.6 ± 0.78	3	5.3 ± 0.85	5	14.4 ± 1.06

n: Sample size; data are expressed as: mean ± SD.

The carp and eels probably lose the same amount of blood as goldfish, however, the loss was negligible because of their larger body size and at least 10 times larger amount of blood. It is concluded that this osmometer system will have many uses in experimental biology involving even smaller animals than fishes, and will also be clinically applicable to examining such samples as children's blood, lymph, edema fluid, tissue fluid, etc.

1 This study was supported in part by Research Grant 387036 from the Ministry of Education of Japan.
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Conduction velocity of peristaltic waves in the in vivo ureter: application of a new diameter gauge

T. Ohhashi, T. Miyazawa, T. Azuma and M. Sakaguchi[1]

Department of Physiology, and Research Laboratory for Cardiovascular Diseases, Shinshu University, School of Medicine, Matsumoto (Japan 390), 4 August 1980

Summary. The conduction velocity of peristaltic movements of the canine ureter was measured under anaesthesia with a new type of diameter gauge using an image sensor. The peristaltic velocity was 34.1 ± 6.2 mm/sec in 10 experiments. Noradrenaline at a low dosage of 1 μg/kg i.v. reduced the resting diameter, increased the conduction velocity to 47–56 mm/sec, and approximately doubled the frequency of contraction. The application of acetylcholine also caused an increase in both frequency and conduction velocity (42–46 mm/sec). A plot of the conduction velocity against the mean period of peristaltic contraction was hyperbolic in shape.

The activity of the ureter has been assessed in various ways, i.e., by measuring the conduction velocity, the frequency of peristaltic waves, or the changes of circumferential or longitudinal tension of the wall[2-5]. Such information cannot be acquired without causing some mechanical disturbance to the ureter like intubation for pressure measurement, or without making direct contact with its outer surface. We have recently constructed a diameter gauge utilizing the high resolving power and stability of the image sensor[6]. The device makes it possible to carry out contact-free diameter measurements of a cylindrical organ.

The purpose of this paper is to describe the use of 2 diameter gauges placed underneath the canine ureter in vivo to measure the peristaltic velocity and to study the effects of adrenergic and cholinergic agonists on the activity of the ureter.

Materials and methods. 15 mongrel dogs of both sexes, weighing 10–16 kg, were anaesthetized with pentobarbitone sodium (Nembutal, Abbott), 30 mg/kg i.v. A tracheal cannula was inserted, and the animal was ventilated with a Harvard respiration pump (Type 613). A femoral vein was cannulated with a polyethylene catheter for infusion of a physiological saline solution (infusion rate: 100 ml/h) and injection of drugs. The ipsilateral femoral artery was cannulated with a polyethylene catheter to record systemic arterial blood pressure by means of a pressure transducer (Toyo Baldwin MPU-0.5-290).

The left ureter was exposed from the renal pelvis to the bladder using a Flank approach into the retroperitoneum. The 2 diameter gauges were positioned at the proximal and distal portions of the exposed ureter. Conduction velocity of the contraction wave was calculated from the distance between the 2 gauges and the time interval between the onset of diameter reduction at each of the 2 positions. The construction and characteristics of the diameter gauge have been described elsewhere[6].

The drugs used were dl-noradrenaline hydrochloride (Sankyo Ltd) and acetylcholine chloride (Daiichi Seiyaku Ltd). All drugs were freshly prepared in saline and administered i.v. All doses are expressed as the weight of base per b.wt (μg/kg).

Results and discussion. Figure 1 demonstrates a typical in vivo recording of ureteral diameter changes at 2 positions. The diameter at the distal position (D2) first increased slightly, and then began to decrease 0.5 sec after the diameter at the proximal position (D1) had started to reduce. The distance between the 2 positions was 18 mm giving a conduction velocity for the peristaltic wave of 36 mm/sec. The average value of the velocity and its SE were 34.1 ± 6.2 mm/sec in 10 experiments.

In order to minimize the effect of contact, Constantinou et al.[7] recorded spontaneous action potentials of the ureters of dogs in vivo with very delicate, loosely attached electrodes. The average wave velocity thus obtained was about 45 mm/sec (20–60 mm/sec). Electrical changes other than the action potentials have often been included in records taken from the in vivo ureter, in dogs and human beings. These changes occurred in association with the passage of urine through the ureter[8]. In some cases, therefore, it is not easy to evaluate accurately the conduction velocity of the in vivo ureter by using the electrical signals recorded with bipolar electrodes.

Effects of noradrenaline and acetylcholine on the conduction velocity of the in vivo canine ureter were then examined. Noradrenaline (NA) was administered i.v. at a dosage of 1 μg/kg i.v. (figure 2, A). NA reduced the resting diameters at the 2 positions, increased the conduction velocity up to 47–56 mm/sec, and approximately doubled

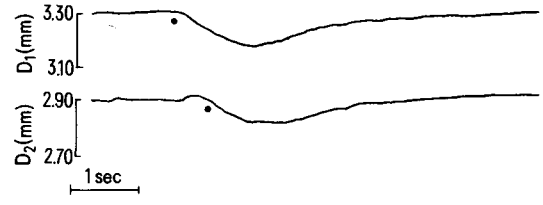

Fig. 1. 2 peristaltic diameter changes of the in vivo canine ureter. D1 and D2 show the diameter changes at the proximal and distal positions, respectively. Dots indicate starting points to reduce each diameter. The distance between the 2 positions is 18 mm.