OCV of the preparations incubated in MEM, we could see that the introduction of this solution was followed by a gradual increase of the SCC and OCV values (preceeded, in frog skin and isolated epithelium, by a sharp, transient fall shown in Figures 1 and 2). In the experiments with whole frog skin, the upwards trend of the SCC continued for the next 10–12 h, after which a plateau was kept at a level of 16–17 $\mu A/cm^2$, which was 3–4 times higher than the control preparations at the same time, and twice as the initial values obtained with this structure. Open circuit voltages increased from 28 $\pm$ 2 mV at the outset of the experiments to 57 $\pm$ 3 mV at 20 h (in control preparations, OCV at 20 h was 13 $\pm$ 3 mV).

In the group of isolated epithelium of frog skin incubated in MEM, the SCC also increased steadily during the first 8 h, reaching a mean value of 12 $\mu A/cm^2$, which is more than twice the value obtained in the control group at the same time (Figure 2). After this period the SCC tended to decay, but the mean value at 20 h was still 8.6 $\mu A/cm^2$, in contrast with the almost complete deterioration of the control preparations.

In the case of the toad bladders, the very low initial values of the SCC increased very quickly after the introduction of MEM, the mean value reaching a peak of 15 $\mu A/cm^2$ at 4 h of incubation. From this time on, there was a downwards trend of the SCC, which tended slowly towards a plateau-like region at 8–9 $\mu A/cm^2$. The voltage values were initially very low (3 mV), rising to 10 mV at 2–4 h and keeping this value for the remaining period.

Stimulation by antidiuretic hormone. The difference between the control and experimental groups was also manifested in the intensity of the response to antidiuretic hormone. In fact, preparations incubated in MEM reacted to the addition of this hormone with an increase in the SCC which was of the order of 20 $\mu A/cm^2$ for whole frog skin, 10 $\mu A/cm^2$ for the isolated epithelium and 9 $\mu A/cm^2$ for toad bladder. In comparison the increase of the SCC in control groups was transitory and with a mean peak value of 7 $\mu A/cm^2$ for whole frog skin, and was negligible for frog skin epithelium and toad bladder. The degree of response to antidiuretic hormone in the experimental groups may also be compared with the results obtained in a previous work with preparations incubated in Ringer where the hormone was used at an early stage (4–6 h). The increase of the SCC was of the order of 15 $\mu A/cm^2$ for whole frog skin and 12 $\mu A/cm^2$ for the separated epithelium[7] and 9 $\mu A/cm^2$ for toad bladder (unpublished results).

In summary, considering that the SCC of frog skin and toad bladder is a function of the net sodium flux[2,8], these results show that there is a definite improvement in the sodium transport capacity of these structures when MEM is used. This improvement is patent either in the first phase of the experiments, when the increase in the SCC in the preparations incubated in MEM contrasts with the tendency to decay of the control ones, and in the long-term maintenance of the electrical parameters and the capacity to react to a physiological stimulus. Morphological studies should help to elucidate the effects of MEM in conserving the transport capacities of these isolated tissues.

Summary. The sodium transport capacity across frog skin and toad bladder in vitro is enhanced when a tissue culture medium is used to mount the preparations, instead of the classic Ringer solution. The response to antidiuretic hormone used 24 h after isolating the tissues is also higher in preparations incubated in the same culture medium.

M. G. EMÍLIO, M. BALLS[9,10] and H. MENANO[11]

Centro de Biologia, Gulbenkian Institute of Science, Oeiras (Portugal), and School of Biological Sciences, University of East Anglia, Norwich (England), 23 June 1975.

[7] M. G. EMILIO and H. P. MENANO, Biochim. biophys. Acta *382*, 344 (1975).
[8] A. LEAF, J. ANDERSON and L. PAGE, J. gen. Physiol. *41*, 657 (1958).
[9] Present address: Dept. Human Morphology, University of Nottingham, England.
[10] M. Ball's development of amphibian organ culture methods was in part supported by Unilever Research, Ltd.
[11] This work was done with the technical help of Ms. WIVI SVENSSON and M. O. FERREIRA.

A Study of Micro-Circulation in Web of Frog (*Xenopus laevis* Daudin) by Using Laser Doppler Microscope

A method has been described to measure flow profiles in microscopic transparent tubes by the application of a laser technique (Laser Doppler microscope[1–4]). The method yields frequency distributions of flow velocities at various distances from the centre of the tube, An example of such a measurement on biological object is reported here. The velocity profile deviates slightly from a Newtonian parabolic profile.

A frog (*Xenopus leavis* Daudin) lay anesthetized in a shallow lucit tray containing a 0.1% urethane solution. A hind limb was hung up in the air and sustained with metal pins on a holder plate standing perpendicularly to the passage of the light beams. The holder plate had a small window through which the 2 laser beams were brought into the web. Both the tray and the holder were placed on a manipulator which was movable by the scale of 5 μm over a wide range (Figure 1). The greater part of the web and hind limb sustained, except the portion of observation (i.e. the passing area of the beams), was covered with a piece of wet gauge in order to prevent the frog from drying up. Although the breathing via the lung of the frog was not maintained artificially, it lasted throughout the experiments for 3 h under the above conditions.

Measured results of the blood flow velocity in a venule having a diameter of 70 μm are shown in Figure 2, where the ordinate and abscissa indicate the flow velocity of the blood and the appearing number of each velocity, respectively. The peak flow velocity decreased gradually

[1] H. MISHINA, T. ASAKURA and S. NAGAI, Optics Commun. *11*, 99 (1974).
[2] H. MISHINA, T. USHIZAKAS, S. TOKUI and T. ASAKURA, Bull. Res. Inst. appl. Electr. *26*, 51 (1974).
[3] H. MISHINA and T. ASAKURA, Appl. Phys. *5*, 351 (1975).
[4] H. MISHINA, T. KOYAMA and T. ASAKURA, Appl. Optics, *14*, 2326 (1975).

as the probing point was moved stepwise with an interval of 5 μm from the central portion of the venular vessel toward its wall. Figure 3 shows the relationship between the peak flow velocity and the probing point. The flow rate through the venule could be estimated if the velocity profile was numerically integrated, assuming the venular vessel to be a cylinder having a diameter of 70 μm. The flow rate so obtained was 2.41×10^{-6} ml/sec. By using this value, a hypothetical parabola flow profile was evaluated with the assumption of a Newtonian flow. The result is plotted in Figure 3 with a broken line which shows a difference from the velocity profile experimentally obtained (centerline velocity higher by 12%).

GAEHTGENS et al.[5] using glass capillaries and a double-slits microscope technique showed that the profile of the capillary flow was markedly blunt in comparison with the parabolic one. However, BAKER and WAYLAND[6] suggested that the blunting effect observed in their measurements was caused by averaging all the correlation signals produced at every point of the light path within the capillary under the observation by a microscope, and

[5] P. GAEHTGENS, H. J. MEISELMAN and H. WAYLAND, Microvasc. Res. *2*, 13 (1970).
[6] M. BAKER and H. WAYLAND, Microvasc. Res. *7*, 131 (1974).

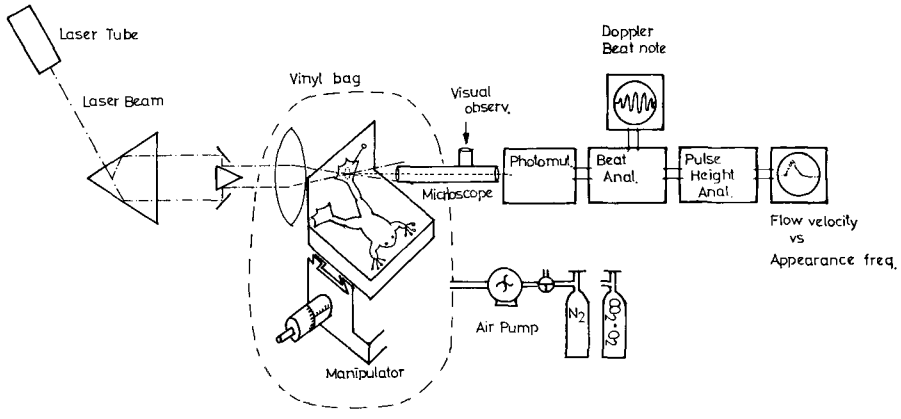

Fig. 1. A schematic illustration of the Laser Doppler microscope together with an experimental arrangement.

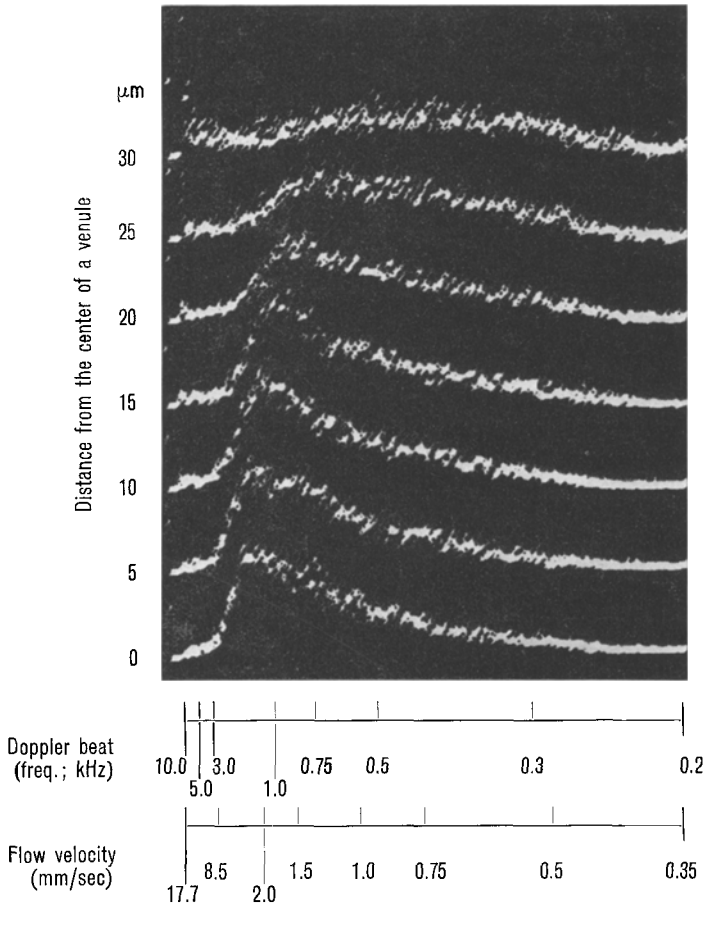

Fig. 2. Frequency distribution of the blood flow velocity in a venule. The probing point was moved stepwise with an interval of 5 μm from the centre of the flow toward the wall.

estimated that the profile shapes in glass tubes above the 40 μm diameter was nearly parabolic at all $\overline{U}$ (average tube velocity/diameter) above $\overline{U} = 6\ sec^{-1}$. On the other hand, the crossed-beam Doppler method employed in the present study has the advantage that the beat signal is obtained only in the clearly defined small volume (10 μm in the cross-section diameter and 15 μm in the depth) in which the 2 incident beams are crossed. The results obtained by using that method is expected to be little affected by the process of averaging the whole profile. The present measurements were performed in the venule in vivo and the estimated $\overline{U}$ is 9 sec^{-1} nearly equivalent to the critical value of 6 sec^{-1} below which the flow profile changes according to BAKER and WAYLAND. Thus, the velocity profile in the venule seems to deviate slightly from the Newtonian flow. In the frog venule, a pulsatile blood flow was also often observed and the periodic changes of flow velocity were weaker than those in mammalian mesentric venule[7]. The present measurements were made in a venule where the pulsatile changes of flow velocity was almost undetectable.

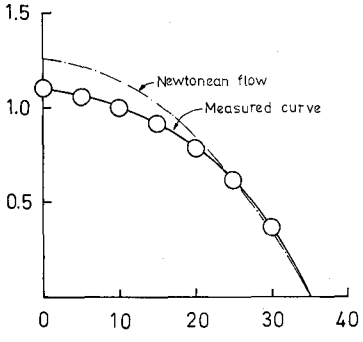

Fig. 3. A profile of the blood flow velocity in the venule. Peak velocity, obtained from Figure 2, on the abscissa against the distance of the probing point from the centre of the flow. The broken line indicates a parabolic flow for the flow rate estimated from the flow profile experimentally obtained.

Finally it must be mentioned that the response and reproducibility of the present method were ascertained through measurements of the capillary blood flow on a repetition of exposing the frog web to normoxic (20% O_2), hypoxic (0.5% O_2) and hypercapnic (80% CO_2) gas mixtures. The broken line in Figure 1 indicates a vinyl bag covering the frog and a part of microscope, into which the above gas mixtures were blown. The output signals of the microscope showed a clear decrease of the blood flow velocity by exposing the web for 6 min to both the hypoxic and hypercapnic gases. Once the gas in the bag was replaced with normoxic gas, the signals showed a smooth recovery of the blood flow velocity to the initial level.

Summary. The flow velocity profile in the venule of frog web was measured by using a laser Doppler microscope of a crossed-beam which permitted a measurement of flow velocity in a clearly defined small volume. The flow velocity profile in the venule seems to deviate slightly from the Newtonian parabola.

T. KOYAMA, H. MISHINA and T. ASAKURA

Research Institute of Applied Electricity, Hokkaido University, Sapporo (Hokkaido, Japan), 13 June 1975.

[7] P. GAEHTGENS, Pflügers Arch. ges. Physiol. *316*, 140 (1970).

Noradrenaline Synthesis in Human Fetal Heart[1]

The adrenergic system is important for the regulation of the fetal circulation[2], for the maintenance of the cardiac function under conditions of asphyxia[3], and for the adaptation of the fetal cardiovascular system to extrauterine life[2]. Noradrenaline-containing nerve terminals have been visualized by the histochemical method of Falck and Hillarp in human fetal hearts from the 11–12th gestational weeks the innervation starting in the atria and reaching the ventricles a few weeks later[4]. Hitherto, it has been uncertain whether the noradrenaline in the human fetal heart is of extra-cardiac origin extracted from the circulating blood or is synthesized within the adrenergic components of the heart itself. This study investigated the capacity of the human fetal heart to synthesize noradrenaline.

Material and methods. 6 fetal hearts from human fetuses (crown-heel length 11–23 cm) were obtained from therapeutic interruptions of pregnancy by laparotomy. The hearts were dissected and placed in a modified Krebs-Ringer solution containing glucose 3.3 m*M* and low molecular weight dextran 2.5% (w/v), aerated with 95% O_2 and 5% CO_2 giving the solution a pH of 7.35 ± 0.06. A coronary perfusion via aorta was started within a few min after the interruption with the Krebs-Ringer solution, and the hearts were placed in a thermostated (37 ± 0.2 °C) jacketed glass bath. After an equilibration time of 15 min, the perfusion was changed to the following solution: To 200–400 ml of the Krebs-Ringer solution was added 10-20 μCi L-tyrosine-C^{14} (243 mCi/m*M*) and 3.7 mg nonradioactive L-tyrosine (in experiment D, 18.1 instead of 3.7 mg L-tyrosine was used). Shortly before

Table I. Data of the human fetal hearts

Case	Gestational age (weeks)	Fetal crownheel length (cm)	Rate of perfusion (ml/min)	Heart activity during perfusion
A	13	11	2.7	normal
B	14	11	1.2	slow
C	16	16	2.3	normal
D	19	20	4.4	normal
E	20	22	6.3	normal
F	23	23	4.0	normal

[1] This study was in part supported by grants from the Hulda Almroth Foundation and the Ferrosan Jubileum Foundation.

[2] G. S. DAWES, *Foetal and Neonatal Physiology* (Year Book Publ., Chicago 1968).

[3] S. E. DOWNING, T. H. GARDNER and J. M. ROCAMORA, Am. J. Physiol. *217*, 728 (1969).

[4] G. GENNSER and C. OWMAN, unpublished observation.