was restrained by a damp gauze wrapping upon a styrofoam board. Bright white light from Ortholux tungsten lamp was focussed within the pupillary area of the eye and regulated by a mechanical shutter. ERG's were displayed upon a Tectronix oscilloscope and filmed on photographic paper. As a test that recording techniques were not generating unusual artifacts, ERG's were recorded from frogs *(Rana pipiens)* in similar fashion immediately before the cichlid recordings. Figure 2 shows 2 consecutive recordings, the first of a frog and the second of a cichlid, with same recording, stimulation and adaptation states. The negative response clearly dominates the cichlid recording.

This negative response of the cichlid retina, dominant in the local ERG elicited by long duration stimuli, does not match readily with known retinal responses. At first approximation, the cichlid negative response might be the teleost version of the PNR; however, the PNR is not considered a dominant contributor to the gross retinal currents recorded externally[2]. In addition, its potential change is positive upon stimulus cessation, while the PNR possesses on- and off-responses of similar polarities. The cichlid response is relatively indifferent to stimulus orientation and configuration, and is apparently quite labile to repeated stimulation. The cichlid negative response must therefore be considered at least a highly modified variant of documented retinal responses, and in view of the high degree of organization of the cichlid retina[3], may reflect an integrative field potential generated within the distal retina. The potential is not exceptionally amenable to investigation, since fish cannot be anesthetized and extremely low (ca. 50–75 μV) potentials are recorded; however, the occurrence of this retinal response in a family of teleosts well-known for their exceptional dependence upon visual stimuli suggests that the visual analysis of these fishes might provide a significant contribution to the interpretation of information processing in the vertebrate retina.

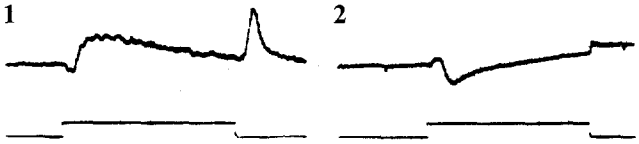

Fig. 2. Responses of frog and cichlid eye to similar stimuli. Sweep speed is 2 sec, vertical scale is 50 μV cm^{-1}, bottom trace is stimulus duration.

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Reduced deformability of erythrocytes exposed to hypercapnia

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Summary. The effect of hypercapnia on the deformability of erythrocytes was studied by means of a nuclepore membrane filter method. A decrement of the deformability by 20–40% was observed when P_{CO_2} was increased from 50 mm Hg to 200 mm Hg, accompanied with an increment of 5% in hematocrit value.

Erythrocytes absorb water and swell, when exposed to hypercapnia[1]. The deformability of the swollen erythrocytes may be lowered, which would act to reduce capillary blood flow. This consideration motivated us to measure the deformability of the erythrocytes which were exposed to carbon dioxide.

The nuclepore membrane filter method of Reid et al.[2, 3] was employed with a slight modification. A 3-way valve and an injection syringe containing saline were connected to the outlet beneath the membrane holder. A small amount of saline was introduced by this syringe through the membrane into the vertical syringe above the filter to push out air bubbles. After closing the saline containing syringe by the valve, a blood sample of 0.6 ml was gently introduced onto the saline on the bottom of the vertical syringe. The time required for 0.5 ml of sample blood to pass through a membrane with 5-μm pores under a pressure difference of 10 cm H_2O was measured. The flow rate (ml/min) thus obtained was considered as a measure of the deformability.

Fresh venous blood (21 ml) obtained from each of 6 healthy subjects was anticoagulated with 1000 units of heparin solution (1 ml) and divided into 3 10-ml injection syringes. Carbon dioxide (2 ml) was introduced into 1 injection syringe. This syringe was slowly rotated in a water bath at 37 °C for 5 min. The blood in the 2nd syringe was not exposed to any external gas and served as a normal control. The blood in the 3rd syringe served to confirm the reliability of the present measurements; the known effect of hyperosmolarity on the deformability of erythrocytes was examined. For this NaCl was added (2.8 mg/1 ml blood), which resulted in an elevation of the osmotic pressure from the normal value of 300 mOsm to about 440 mOsm.

Erythrocyte deformability and other physiological parameters in 6 subjects (mean value ± SD). The differences between normal and experimental measurements were significant by Student's paired t-test ($p < 0.01$)

Condition	Hct (%)	pH	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Deformability (ml/min)	Ratio of deformation
Normal	40.6 ± 2.2	7.356 ± 0.037	62.0 ± 14.3	51.5 ± 5.9	2.72 ± 0.32	1
$+CO_2$	42.4 ± 2.3	6.909 ± 0.049	83.8 ± 18.8	195.7 ± 15.9	1.95 ± 0.37	0.72 ± 0.09
+NaCl	35.0 ± 2.9	7.354 ± 0.025	48.6 ± 7.1	48.8 ± 4.8	0.43 ± 0.16	0.16 ± 0.07

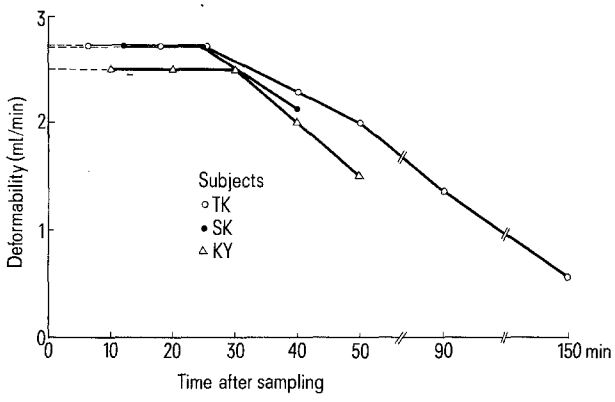

Fig. 1. Changes of deformability (flow rate) of the control blood samples in 3 subjects as a function of time after blood sampling.

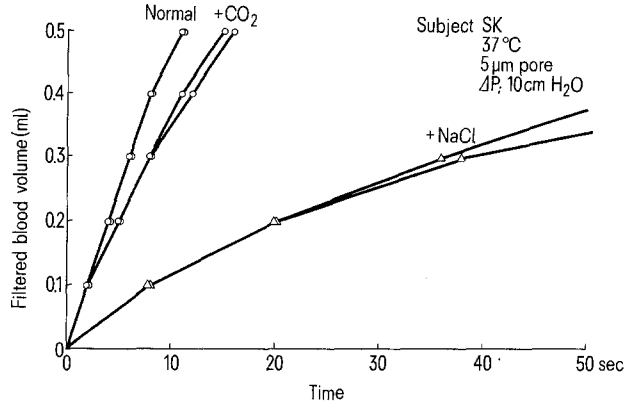

Fig. 2. An example of deformability measurement on 3 kinds of blood samples. Flow rate of each sample was sequentially measured twice within 30 min after blood sampling.

The deformability measurements on the 3 samples were sequentially carried out twice within 30–40 min (figure 1) after obtaining the blood. Then P_{O_2}, P_{CO_2} and pH of each sample were measured by means of a Radiometer model BGA-3 gas analyzer. Hematocrits of the 3 samples were measured at the same time. All the measurements were finished within 2 h after sampling.

The deformability (flow rate) of the normal control blood of 3 subjects was repeatedly measured to ascertain the reproducibility of the measurements. The results obtained are plotted against the time-lapse after sampling (figure 1). The deformability was unexpectedly unstable and remained constant only for 30 min after sampling. Since the deformability measurement had to be made in such a short time period, only 2 or 3 serial measurements could be made. An example of the measurements is shown in figure 2, where the blood volume passing through the membrane is plotted against the time. The curves representing the 6 measurements on the 3 kinds of blood samples are clearly separated. The rate of blood passage is slightly but clearly reduced in hypercapnia. The same pattern was observed in all the measurements on blood from 6 subjects. The mean values and SD of the deformability are summarized in the table together with other physiological quantities.

Normal control values of the deformability obtained in the present study are 6–20 times larger than those of Reid et al[2,3]. The dispersion of our values is less than that of theirs. These discrepancies seem to be attributable to the difference of time length required for the completion of the measurements. Our measurements were made at the most in 40 min, while those of Reid et al.[2, 3] were made in 2 h after sampling. In the present method, the complete removal of air bubbles around the membrane is essential to obtain reproducible results. The present modification seems to have improved the facility of air-removal. One piece of nuclepore membrane could be used for the serial measurements repeated within 30 min and produced fairly coincident results.

The validity of our values may be supported by the following estimations. The area through which the blood flows is estimated to be 2.4×10^{-2} cm^2 from the effective area of the membrane (0.3 cm^2) and the pore density (4×10^5/cm^2). When a flow rate of 2.7 ml/min is divided by the area, it gives a flow velocity of 22 mm/sec through the pores. Since the length of the pores is 10 µm, the pressure gradient is 10^4 cm H_2O/cm. On the other hand, the blood flow velocity in the human capillary vessels is 0.5–1 mm/sec and the pressure gradient is 200–400 cm H_2O/cm. The ratio of the flow velocity to the pressure gradient is nearly the same in both cases.

The reduced deformability in hyperosmolarity further confirms the usefulness of the present method. Moreover, the deformability decreased by 80% in response to elevated osmolarity is clearly larger than the decrease reported by Schmid-Schönbein et al.[4] for a comparably elevated osmolarity. This quantitative difference is probably due to the difference in the time lapsing after blood sampling. Their method required a longer preparation time and made up to 72 h after obtaining the blood[4].

In the studies on hypercapnia, the P_{CO_2} of the blood was increased from 50 mm Hg to 200 mm Hg (table). The increment of the volume of erythrocytes was estimated to be about 5% from the hematocrit measurement, while the decrement of the deformability was 20–40%. The large decrease in the ability to pass through 5-µm pores cannot be explained by the volume-increment alone. The deformability of erythrocytes also depends on cellular metabolism[5]. Carbon dioxide may affect the metabolic state and stiffen the cell membrane. In any case, the decreased deformability will oppose the vasodilatory effect of hypercapnia and interfere with the microcirculation. This could be important in tissues which are exposed to ischemic hypercapnia[6]. Since the deformability measured by the nuclepore filter method may include the viscosity of the substance contained in the erythrocytes and the flexibility of the erythrocyte membrane, the interpretation of the values obtained may be difficult. However, the advantages of the nuclepore filter method are the uniform pores of the filter and the short time required for the measurements. It was only due to these improvements that the reduced deformability could be observed in blood exposed to the hypercapnia.

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