Rhythmic Wavelets Recorded from an in vitro Preparation of Mammalian Retina

In 1953, COBB and MORTON observed that human electroretinograms showed several rhythmic wavelets superimposed on the ascending phase of *b*-wave[1]. This finding has been confirmed by BORNSCHEIN and GOODMANN[2], HECK and RENDAHL[3] and subsequently by many others. It has been referred to as oscillatory potential by YONEMURA et al.[4].

Recording of electrical activity from mammalian retina has made great progress recently. The most crucial problem in this field of study is the difficulty to keep the excised retina alive in physiological state in vitro. As compared with the retina of cold-blooded animals, that of mammals is far more fragile for surgical procedures.

In 1960, AMES and GURIAN have succeeded in keeping it alive and recording *a*-, and *b*-waves[5,6]. SICKEL et al.[7], HANITZSCH and BORNSCHEIN[8] and others succeeded in tracing these waves in vitro with each unique method, for the purpose of research on different problems of ERG. In 1963, PAUTLER and WILSON recorded *c*-wave from the excised mammalian retina with intact pigmented epithelium[9]. But attempts to record rhythmic wavelets in vitro have been unsuccessful. In this report, rhythmic wavelets recorded from an in vitro preparation of mammalian retina will be described.

Materials and methods. (a) Biological preparation prior to surgical procedure. White rabbits, weighing about 2 kg were used. An opaque contact lens (Figure 2) was sown on episclera at 4 points with silk suture. After dark adaptation for about 2 h, subsequent manipulations were performed under dim red light at room temperature (25–30 °C).

(b) Anesthesia. 3 min prior to operation, instillation anesthesia was induced with 2 drops of 4% Xylocain (Lindocaine Hydrochloride). Immediately before operation, conjunctival sack was washed by physiologic saline solution to remove the excess anesthetics. No general anesthesia was given.

(c) Operation. Lateral and medial canthus were cut off. Conjunctiva was incised about 3 mm from the edge of contact lens and was reflected to expose extraocular muscles. Then the muscles were cut one after another. During these procedures, vortical vessels were not damaged and bulbus was not rotated but fixed by placing the situation mark of contact lens at the right position. The eye was not pulled outwards. When whole sclera was ready to be removed, the vessels and optic nerves were cut finally with scissors. The excied bulbus was immersed in an incubation medium (see below) and was opened at the equator as rapid as possible using a sharp blade. It took approximately 5 sec to open the bulbus. The vitreous and anterior portion of the bulbus were then separated from the posterior portion composed of retina, choroid and sclera.

(d) Incubation medium. Ames solution was used (AMES and HASTINGS[10]). The solution was similar to human cerebrospinal fluid in its electrolyte pattern and contained 10 m*M* of glucose/l. The medium was equilibrated by bubbling 95% O_2 and 5% CO_2 for more than 5 min. The flow rate of gas was set 2 l/min. No blood plasma was added to the medium, nor hyperpressure given.

(e) Temperature. All manipulations mentioned above were carried out at 30 °C. The temperature of incubation chamber was maintained at 35 °C in a water bath (Figure 1). It was assured by a needle thermister probe.

(f) Electrical procedure. A pair of Ag-AgCl meshwork electrodes (1.19 mm square) were used. The electrodes were located in the center of septum of a reaction chamber. They conform to the curvature of eyeball as shown in Figure 3. The area of retina exposed to light was 10 mm in diameter, and the other field was shielded from light. The retina preparation was held between the electrodes in constant pressure by 2 insulated pins, and was inserted to the reaction chamber as shown in Figure 1. The electrodes connected to an universal dual beam oscil-

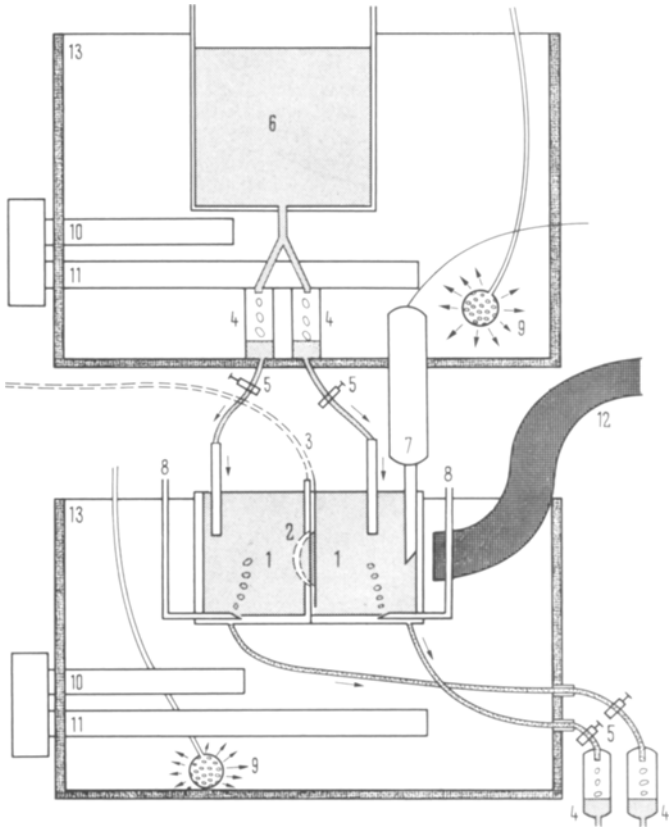

Fig. 1. Principle of incubating apparatus: 1, reaction chamber. For the sake of explanation, it was drawn relatively enlarged in this Figure. 2, septum with meshwork electrodes and preparation in between. It was inserted into the slit of chamber. 3, shielded leading wires connected with an universal dual beam oscilloscope. 4, droppers. They were necessary for the circulating medium of each chamber to be insulated. 5, cocks for controlling the flow rate of medium. 6, a depot of medium. 7, thermister needle to monitor the temperature of medium from outside of shielded room. 8, nozzles for bubbling the medium with a mixture of 95% O_2 and 5% CO_2. 9, agitator of water bath. 10, thermostat. 11, heater. 12, fibre optics. Stimulating light was guided to chamber by it. 13, water bath. A pair of baths were prepared for temperature control of medium.

[1] W. A. COBB and H. B. MORTON, J. Physiol. *123*, 36 (1953).
[2] H. BORNSCHEIN and G. GOODMANN, Archs Ophthal., N.Y. *58*, 431 (1967).
[3] J. HECK and I. RENDAHL, Acta physiol. scand. *39*, 167 (1957).
[4] D. YONEMURA, K. TSUZUKI and T. AOKI, Acta ophthal. Suppl. *70*, 115 (1962).
[5] A. AMES and B. GURIAN, J. Neurophysiol. *23*, 671 (1960).
[6] A. AMES and B. GURIAN, Archs Ophthal., N.Y. *70*, 337 (1963).
[7] W. SICKEL, H. G. LIPPMAN, W. HASCHKE and CH. BAUMANN, in *The Visual System* (Springer, Berlin 1961), p. 80.
[8] R. HANITZSCH and H. BORNSCHEIN, Experientia *21*, 484 (1956).
[9] E. L. PAUTLER and A. R. WILSON, Vision Res. *3*, 507 (1963).
[10] A. AMES and A. B. HASTINGS, J. Neurophysiol. *19*, 201 (1956).

loscope through preamplifiers with various time constants. The electrode attached the retina was connected to positive deflection, which was recorded upwards in oscilloscope, and that attached to the sclera was negative deflection.

(g) Incubation chamber. The chamber was made of transparent methyl methacrylate. It was separated into 2 parts by the septum inserted as described above. Each part contained the medium in a volume of 20 ml. The chambers were circulated by the medium at a constant speed of 2 ml/sec respectively, and were aerated continuously with a mixture of 95% O_2 and 5% CO_2 through the nozzles located at the bottom of each chamber.

(h) Stimulation. The principle of photo stimulator employed in this study was the same as that reported by NAGATA and JACOBSON[11]. A tungsten lamp of 1000 W for slide projector was used for a luminescent source. The light intensity to stimulate the retina was varied from 5–10,000 lux (in logarithmic scale, from 0.7–4.0) at the surface of the retina, when measured in air, for convenience. Square wave light stimulus emitted from the stimulator was guided by a fibre optics to the chamber. Interval of the stimulus was 0.1 c/sec. The retina in reaction chamber was given a rest for 2 min before the start of stimulation. During a limited period for photographic recordings, the gas flow was stopped.

Results and discussion. Figure 4 represents a typical ERG obtained from an in vitro preparation of rabbit retina. In this Figure, dominant *a*-, and *b*-wave of relatively low voltage, as compared with those in vivo, and ascending phase of *c*-wave are shown. Here, considerable attention must be given to the ascending phase of *b*-wave. There are distinct 4 rhythmic wavelets, following a_1-, and a_2-waves. This is a new finding on mammalian ERG in vitro. Frequency of these 4 wavelets is about 200 c/sec,

[11] M. NAGATA and J. H. JACOBSON, in *Proceedings 3rd ISCERG Symposium* (Pergamon Press Inc., London 1964), p. 235.

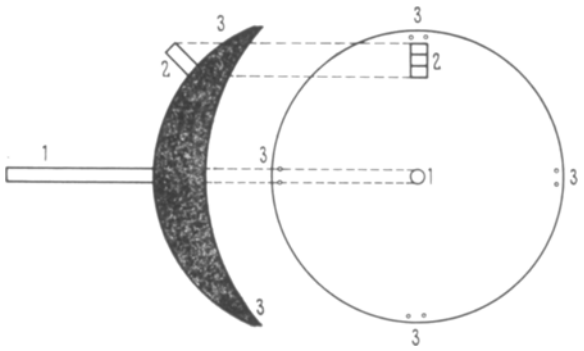

Fig. 2. An opaque contact lens. It was sown on episclera prior to start of dark adaptation. 1, handle for manipulation. 2, situation mark. 3, aperture for suture.

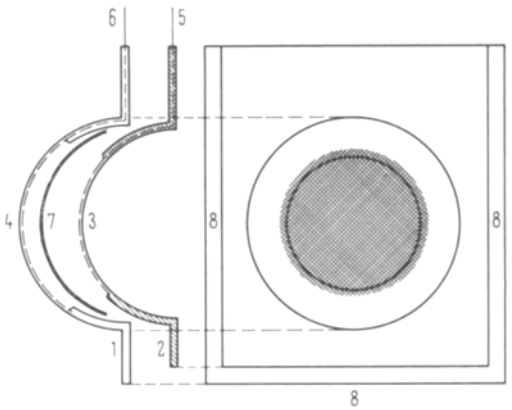

Fig. 3. Septum of chamber with electrodes. 1, main septum. 2, accessory septum. The area exposed to light was 10 mm in diameter and the other field was shielded from light. Each septum with a preparation in between were held in constant pressure. 3, 4, Ag-AgCl meshwork. 3, was connected to positive deflection and 4 to negative deflection through shielded wires of 5 and 6. 7, retina attached with choroid and sclera. 8, narrow area inserted into the slit of chamber.

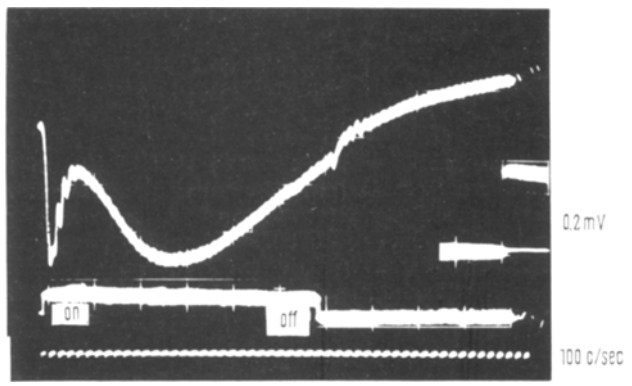

Fig. 4. A typical tracing obtained from an in vitro preparation of a rabbit retina. This photograph is taken at the time of 30 min after enucleation. Time constant is 0.3 sec. Upward deflection on the middle tracing represents duration of stimulus. Log I, 2.5. Calibration, 0.2 mV. Time mark, 100 c/sec.

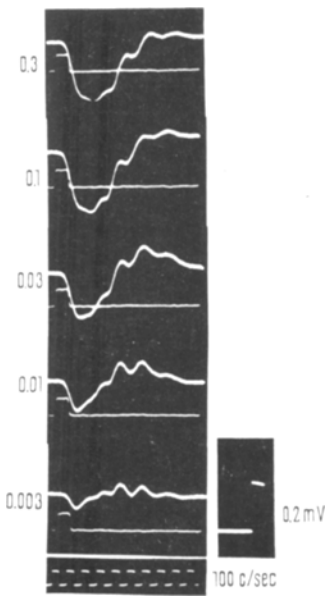

Fig. 5. Change in pattern of rhythmic wavelets recorded through the channels with various time constants. Numbers on the left side represent time constants of channels in sec. This tracing was obtained 10 min after enucleation. Log I, 2.5.

and is approximately equal to that of oscillatory potential on rabbit ERG in vivo. Incubation periods of these wavelets are varied, according to intensity and duration of stimulus, time elapsing after enucleation and so on, although frequency is constant. Changes in pattern of rhythmic wavelets, recorded through the channels with various time constants, are represented in Figure 5. Life-span of these wavelets on continuous observation is shown in Figure 6. On the tracing at 180 min and subsequent one, they disappeared presumably as a result of inadequate supply of nutrition. On the tracing at 120 min, there still remain wavelets although diminishing in amplitude and number, but no dominant *b*-wave is recorded. Comparative disappearance of *b*-wave at this period hereabout was assured after dark adaptation of 5 min. Descending phase of *a*-wave of many tracings in Figure 6 can be divided into 2 different slopes, anterior steep one and posterior moderate one. Presumably this finding is faithfully reflected on the inference that this phase depends on the summation of different 2 PIII components, photopic PIII and scotopic PIII.

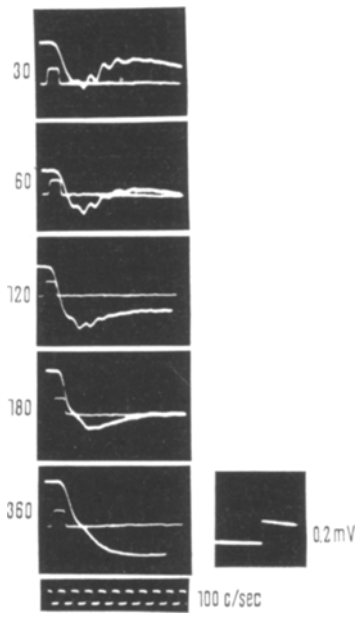

Fig. 6. Life-span of rhythmic wavelets superimposed on the ascending phase of *b*-wave. Numbers on the left represent time elapsing after enucleation (interruption of vasculature in vivo) in min. Log I, 2.5. Frequency of stimulus was set 0.1 c/sec. Time constant is 0.3 sec.

It may be attributable to the following reasons that we succeeded in recording rhythmic wavelets in the present investigation: (1) An opaque contact lens was sown on episclera before dark adaptation. The time elapsing between interruption of vasculature in vivo and immersion of the retina was kept within 5 sec by means of handling an originally designed contact lens. (2) The temperature of the medium for incubation was maintained at 35 °C, which differs from 30 °C or thereabout used by previous authors. The temperature of the medium for operation was also at higher level than that of previous authors[5,12–14]. (3) Flow rate of a mixture of 95% O_2 and 5% CO_2 was maintained at 2 l/min in each chamber of 20 ml in volume. On the other hand, slower flow rate of medium than that of previous authors was employed[7,15]. Both were convenient for retina to be supplied for sufficient O_2. It seemed that the excised retina demands extremely large quantity of O_2 supply compared with that in vivo, especially for the purpose of recording rhythmic wavelets.

Detailed affection of intensity and duration of stimulus, temperature, anoxia and chemical agents toward these wavelets and inquiry of their origin will be described in a subsequent report[16].

Zusammenfassung. Die rhythmischen Potentiale an der aufsteigenden Phase der *b*-Welle des von der In-vitro-Präparation der Warmblüternetzhaut abgeleiteten Elektroretinogramms wurden in vivo dargestellt.

Y. HONDA

Department of Ophthalmology,
Kyoto University, Faculty of Medicine,
Kyoto (Japan), 11 November 1968.

[12] J. BOCK, H. BORNSCHEIN and K. HOMMER, Vision Res. *4*, 609 (1964).
[13] A. LÜTZOW and H. BORNSCHEIN, Albrecht v. Graefes Arch. Ophthal. *170*, 332 (1966).
[14] H. BORNSCHEIN, R. HANITZSCH and A. LÜTZOW, Vision Res. *6*, 251 (1966).
[15] R. HANITZSCH, Albrecht v. Graefes Arch. Ophthal. *170*, 342 (1966).
[16] Grateful acknowledgment is made to emeritus Prof. R. ASAYAMA and Prof. M. KISHIMOTO of Kyoto University, Department of Ophthalmology, for guidance in this investigation. The author is also indebted to Prof. T. ARAKI of Kyoto University, Department of Physiology, for kindly criticism. Thanks are also due to Dr. M. NAGATA, the Head of Ophthalmology, Tenri Hospital, Japan, for helpful suggestions. Dr. A. KANEKO of Keio University, Department of Physiology, is gratefully acknowledged for stimulating this investigation.