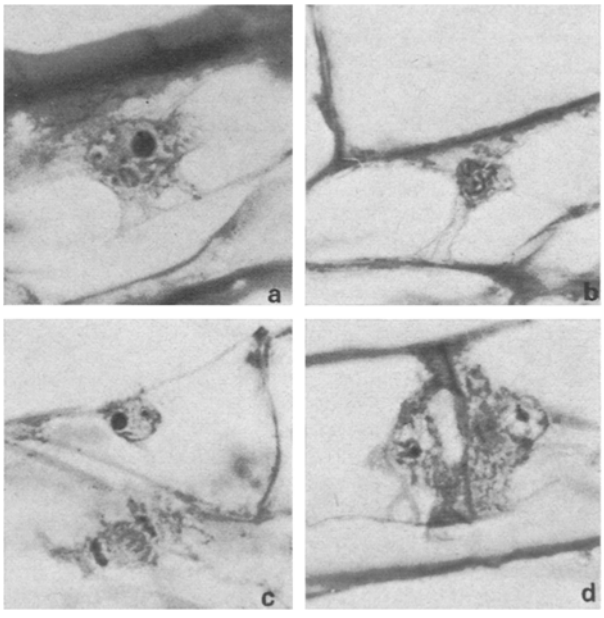
senting all stages of activity were evident in the vesicle stalks incubated on sucrose (treatment b) (figure, a–d). In addition, callus outgrowths were also evident both macroscopically and microscopically in the sucrose-treated explants. This shows that excised juice vesicle stalks from mature lemon fruits are capable of manifesting nuclear and cytoplasmic division when incubated on a single component nutrient medium consisting solely of 4% sucrose. (Note: The possibility cannot be ruled out that exogenous supplies of boron were released to the explants from the borosilicate glass of the 'Pyrex' Petri dishes.)



Mitotic figures from 6-day-old vesicle stalks incubated on 4% sucrose. a) Prophase; b) metaphase, polar view; c) late anaphase plus an interphase nucleus; d) telophase.

The findings presented here demonstrate that mitotic activity can be brought about in excised lemon fruit juice vesicle stalks by supplying the explants with an exogenous source of a sugar which is already naturally present within the sac cells of lemon fruit juice vesicles (see tables 1 and 2 in Kordan[6]). Thus, the evidence presented here supports previous observations[6] which implicate vacuolar physiology as constituting an intrinsic barrier against injury-induced mitosis in lemon fruit juice vesicle cells.

1 I thank the Dr Hadwen Trust For Human Research for funds received in support of this research and Karen Brown for technical assistance.
2 H. A. Kordan, Experientia *30*, 749 (1974).
3 H. A. Kordan and J. Dale, Z. Pflanzenphysiol. *69*, 456 (1973).
4 A. G. E. Pearse, Histochemistry, 2nd ed, p. 788. Little, Brown & Co., Boston 1960.
5 H. A. Kordan, Z. Pflanzenphysiol. *67*, 311 (1972).
6 H. A. Kordan, Nucleus, Calcutta *18*, 109 (1975).

Nature and the time course of the effect of CO_2 on electroretinogram (ERG) in an arachnid

T. Ramakrishna[1,2]

Department of Biophysics, National Institute of Mental Health and Neurosciences, Bangalore (India),
18 November 1976

Summary. Electroretinogram recorded from the median eye of the scorpion, Heterometrus fulvipes before and after exposure to CO_2 indicated that the rate of recovery of 'b' wave to pre-CO_2 level was slow and delayed as compared to 'a' wave. 'b' wave may therefore have a more central origin than that of 'a' wave, which finds corroboration in the results of the depth recording in this eye with the microelectrode.

CO_2 is known to have a pronounced effect on the optic ganglion and hence is known to abolish those components of ERG which arise more centrally during the process of anaesthetization[3]. Though it is known that complex responses are obtained during the initial stages of application of CO_2 and during recovery, neither the time course of these responses nor its significance seems to have been explored fully. The present study is an attempt in this direction in which a simple median eye of an arachnid, the scorpion Heterometrus fluvipes ,has been used, as it is thought that complex processes are relatively easier to understand in simpler systems.

Material and methods. Stimulus assembly. A tungsten filament bulb (12 V, 5.2 W) from a microscope lamp fitted with 2 condenser lenses was the source. Duration of stimulus was controlled through a sectored disc, attached to a DC motor.

Recording devices. Glass pipette electrode (inside diameter 70–80 µm) filled with scorpion ringer[4] was used as the recording electrode (RE). Platinum wire inserted into the glass pipette was connected to the grid G1 of a Grass preamplifier. A steel pin etched electrolytically, and insulated except at the tip, served as an indifferent electrode (IE). Potentials were displayed on a dual beam oscilloscope (Tektronix 502A) and were photographed with a grass C4 camera. Stimulus was monitored through a photocell.

Preparation. Scorpion was restrained with the dorsal side up on a metal base by using plasticene. The surface of the eyes was scraped gently with a microscalpel to remove the wax coat. While the RE was placed over the illuminated eye, the IE was placed over the adjacent unilluminated

1 The equipment was a gift from USAFOSR to Prof. T. H. Bullock and Prof. K. P. Rao, and the work was carried out at the Department of Zoology, Bangalore University. I thank Dr R. V. Krishnamoorthy and Dr A. R. Kasturi Bai.
2 Present address: N. C. Department of Mental Health, Division of Research, Box 7532, Raleigh, N. C. 27611, USA.
3 T. H. Goldsmith, J. gen. Physiol. *43* (4), 775 (1960).
4 B. P. Naidu, Nature *213*, 410 (1967).

eye. As a further measure against light-leakage, the latter was covered with black paper. When the ERG reached a stable level of amplitude, which it did usually after 40–60 min, the experiment was started. All the experiments were carried out in a dark room under a dim red light and at the temperature 26 ± 1 °C.

Process of administering CO_2. A cup of plasticene was built around the scorpion and a small tube inserted through the base of the cup. CO_2 was passed through the tube from Kipp's apparatus. The light-stimulus was allowed to incident over the eye through the open top of the cup.

Results. Normal ERG. ERG in this species has 2 prominent phases: a) a fast rising negative wave (upward deflection), and b) a relatively slow positive wave, and hence is diphasic. The latency measured from the onset of stimulus to the appearance of upward rising negative phase is 70 ± 7 msec. The negative wave ('a' wave) reaches its peak in 61 ± 7 msec. The positive wave ('b' wave) has generally somewhat lower magnitude than the 'a' wave.

Mean values ± SD for the duration (recovery time) in which the amplitude of 'a' and 'b' waves reach their pre-CO_2 levels after turning off CO_2

ERG-component	Recovery time in min
'a' wave	20 ± 0.5
'b' wave	22 ± 0.3

Data were pooled from 9 experiments.

Effect of CO_2. Initial responses. Soon after introducing CO_2, the base line drifted erratically, the amplitude of ERG was greatly reduced. The spontaneous 'spike-like' potentials have appeared, some of which were superimposed over the ERG (figure 1).

Later effects. During the 10-min-period, the CO_2 was allowed to flow over the scorpion, there were fluctuations in the amplitude. In the first 2 min there was 80% reduction in the amplitude of the 'a' wave and 72% reduction in the case of 'b' wave. Later on there was a slight rise in the amplitude of both the waves over the 4th min following another fall over the 6th min. Again there was an increase in the amplitude of both the waves through the 10th min when the CO_2 was turned off. The amplitude of 'a' wave remained constant over the next 2 min, after which there was a further reduction. During the next 4 min the amplitude remained constant. In the case of 'b' wave, however, there was no change during the 8 min following the closure of CO_2. This period of relative stability was followed by a further reduction in amplitude of both the waves, up to the 12th min in the case of 'a' wave and up to the 10th min in the case of 'b' wave. After this period, both the waves gradually increased in amplitude and reached pre-CO_2 levels. While 'a' wave reached the pre-CO_2 level 20 min after turning off of CO_2, 'b' wave reached the same 22 min later (table). In the next 10-min-period, during which the recordings were made, the amplitude of both the waves remained constant. The noteworthy feature of the time course is that

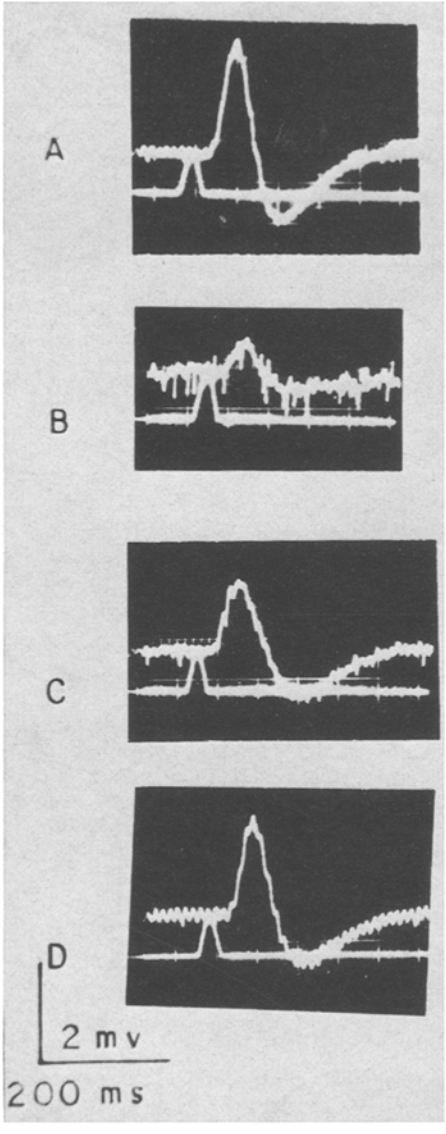


Fig. 1. Oscilloscopic recordings of ERG, made before, during and after CO_2 anaesthesia. *A* Before; *B* 2 min after passing CO_2 (note the spontaneous 'spike-like' potentials some of which are superimposed over ERG); *C* 10 min after closing CO_2; *D* 20 min after. The lower trace in the recordings is the stimulus signal. Intensity used: 100 lux.

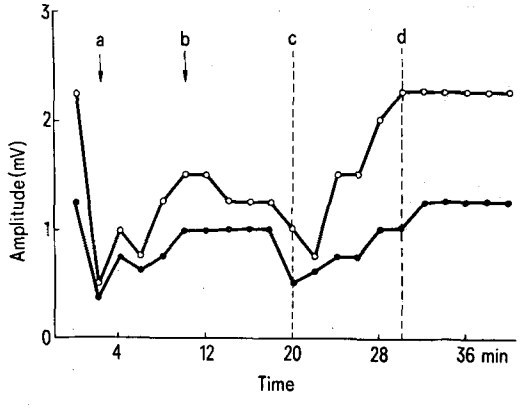


Fig. 2. Time course of changes in the amplitude of ERG from the median eye due to CO_2 anaesthesia, from a typical experiment. ○—○, Changes in negative phase (upward deflection in the oscilloscopic recordings); ●—●, changes in positive phase; *a* CO_2 turned on; *b* CO_2 turned off; *c* recovery; *d* pre-CO_2 level.

10–12 min after turning off CO_2 flow, both the waves show a continuous recovery phase. However, the slope of this phase, marked with broken lines in figure 2, indicates that the 'b' wave had a significantly slower recovery rate than 'a' wave.

Discussion. The effect of CO_2 on ERG in scorpion is somewhat similar to that reported in worker honeybee[3]. However, in this preparation, the 'off' effect of the retinal action potential completely disappeared during the exposure and reappeared after turning off CO_2. Based on this differential sensitivity of the sustained negativity and the 'off' effect, Goldsmith proposed that these 2 waves have different origins. As the effects of CO_2 are pronounced on second and higher order neurons, the 'off' effect which was totally abolished by the same is considered to have a more central origin. Such selective poisoning of certain neural pathways leaving others intact was attempted by other workers, using such agents as cocaine and procaine[5]. While studying the effect of cocaine, Bernhard suggested that the negative component of the retinal action potential in Dytiscus originates in the layer of the retinula cells[6]. Swihart demonstrated that procaine, when introduced into the eye via a corneal hole in Danaus plexippus L. quickly removes positive components from the ERG waveform[7]. This selective action of procaine on the positive components was considered as proof for their origin distal to the basement membrane. In the present results, it is significant that the recovery of 'b' wave not only proceeds at a much slower rate but is also delayed by 2–3 min, when compared to the recovery process in 'a' wave. Even a duration of 2–3 min may be quite significant in terms of electrical events in neural structures. It appears therefore that CO_2 has a more pronounced effect on the 'b' wave and may possibly originate, therefore, at a locus more central to the same from where 'a' wave originates. This conclusion finds corroboration in the results of the depth-recording in this eye with the microelectrode[8]. Thus the present study possibly indicates that the temporal aspects of the effect of exposure to and recovery from CO_2 may be helpful in delineating the peripheral electrical events, even if such exposure does not result in total suppression of one or more components.

5 G. Camougis, in: Psychopharmacology – A Review of Progress, 1957–1967, p. 401. U. S. Public Health Service, Washington, D.C. 1968.
6 C. G. Bernhard, J. Neurophysiol. *5*, 32 (1942).
7 S. L. Swihart, J. Insect Physiol. *18*, 1221 (1972).
8 T. Ramakrishna, Proc. XXVI Int. Congr. Physiol. Sci., October 1974, New Delhi.

Occurrence of 5-hydroxytryptamine in chick retina

O. Suzuki, E. Noguchi, S. Miyake and K. Yagi[1]

Institute of Biochemistry and Department of Ophthalmology, Faculty of Medicine, University of Nagoya, Nagoya 466 (Japan), 6 December 1976

Summary. 5-Hydroxytryptamine (5-HT) was found in chick retina. 5-HT level in chick retina was increased by the administration of pargyline and decreased by reserpine, but remained unchanged with tryptophan.

The existence of catecholamine[2–6], γ-aminobutyric acid[7–10] and acetylcholine[11,12] as putative neurotransmitters in the retinas of several species of animals is well documented. In regard to 5-hydroxytryptamine (5-HT) in the retina, however, comparable information is lacking. While there are a few reports[13,14], including the determination of 5-HT in the retinas of some vertebrates, Häggendal and Malmfors[3] could not detect any 5-HT in rabbit retina. By the histochemical method of Falck and Hillarp, Hauschild and Laties[15] suggested the occurrence of 5-HT in a special type of cells in chick retina. The present paper deals with quantitative analysis of 5-HT in chick retina, and the effects of some drugs on its level.

Material and methods. Chicks of the White Rock breed weighing approximately 1 kg were used. Animals were injected i.p. with pargyline-HCl (100 mg/kg) 2 h, reserpine (5 mg/kg) 5 h or L-tryptophan (200 mg/kg) 2 h before killing. Immediately after decapitation, the eye was enucleated, and the retina was rapidly removed and frozen on dry ice. More than 300 mg of retinal tissue was collected for a sample. 5-HT was analyzed within 24 h after the sacrifice. The fluorometric assay using ninhydrin reaction[16] was adopted for the determination of 5-HT in the retina, for the method is quite specific for 5-HT and excludes the contamination of other indoleamines[17]. The retina was homogenized in 5.0 ml of cold acidified n-butanol[18] by using a glass homogenizer fitted with a Teflon pestle and

1 Correspondence to: K. Yagi, Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya 466, Japan.
2 T. Malmfors, Acta physiol. scand. *58*, 99 (1963).
3 J. Häggendal and T. Malmfors, Acta physiol. scand. *64*, 58 (1965).
4 B. D. Drujan, J. M. D. Borges and N. Alvarez, Life Sci. *4*, 473 (1965).
5 B. Ehinger, Z. Zellforsch. *71*, 146 (1966).
6 S. Nichols, D. Jacobowitz and M. Hottenstein, Invest. Ophthalm. *6*, 642 (1967).
7 K. Kuriyama, B. Sisken, B. Haber and E. Roberts, Brain Res. *9*, 165 (1968).
8 L. T. Graham, Jr, C. F. Baxter and R. N. Lolley, Brain Res. *20*, 379 (1970).
9 M. S. Starr, Brain Res. *59*, 331 (1973).
10 A. Bruun and B. Ehinger, Exp. Eye Res. *19*, 435 (1974).
11 D. M. K. Lam, T. N. Wiesel and A. Kaneko, Brain Res. *82*, 365 (1974).
12 M. J. Neal, in: Transmitters in the Visual Process, p. 127. Ed. S. L. Bonting. Pergamon Press, Oxford 1976.
13 J. H. Welsh, in: Comparative Neurochemistry, p. 355. Ed. D. Richter. Pergamon Press, Oxford 1964.
14 W. B. Quay, Life Sci. *4*, 983 (1965).
15 D. C. Hauschild and A. M. Laties, Invest. Ophthalm. *12*, 537 (1973).
16 S. H. Snyder, J. Axelrod and M. Zweig, Biochem. Pharmac. *14*, 831 (1965).
17 W. B. Quay, J. pharm. Sci. *57*, 1568 (1968).
18 G. Curzon and A. R. Green, Br. J. Pharmac. *39*, 653 (1970).