muscle of guinea pig ileum[7] and in cortical slices from rat brain[8], but it seems to be important to emphasize that there might be an alternative explanation for this effect of ouabain on catecholamine release.

Release of catecholamines has many features in common with the release of acetylcholine from presynaptic terminals[9,10] and it is clear that Ca^{2+} acts as the link in stimulus-secretion coupling in both systems[11,12]. It has also been suggested that the frequency of the spontaneous rate of release of quanta of acetylcholine (recorded as the miniature endplate potentials, MEPPs) at the amphibian neuromuscular junction is largely determined by $[Ca^{2+}]_i$ at the presynaptic terminals[13–17]. Factors that serve to elevate $[Ca^{2+}]_i$ produce an increase in MEPP frequency. Cardiac glycosides also cause a rise in the rate of spontaneous release at the frog neuromuscular junction[18–20] and similar effects are found when Li^+ is allowed to accumulate intracellularly at the presynaptic terminals[21–25]. Such experiments suggest that a rise in the intracellular concentration of either Na^+ (by the action of ouabain in suppressing Na^+-efflux) or Li^+ (which is not readily removed by the cation pump) promotes spontaneous release at the neuromuscular junction and there is now evidence that a rise in $[Li^+]_i$ or $[Na^+]_i$ causes a rise in $[Ca^{2+}]_i$. Such effects have been reported in synaptosomes[26], Chironomus salivary gland cells[27] and in the isolated islets of Langerhans, apparently by release of Ca^{2+} from intracellular stores[28]. It is noteworthy that both Na^+ and Li^+ have been shown to promote Ca^{2+} release from isolated heart mitochondria[29]. It therefore seems probable that the action of ouabain in promoting catecholamine release is also achieved indirectly by a rise in $[Ca^{2+}]_i$ and that one need not necessarily postulate that the Na^+-K^+-ATPase is directly concerned with exocytosis.

7 W. D. M. Paton, E. S. Vizi and M. Aboo Zar, J. Physiol. (Lond.) *215*, 819 (1971).
8 E. S. Vizi, J. Physiol. (Lond.) *226*, 95 (1972).
9 C. J. Duncan, The Molecular Properties and Evolution of Excitable Cells. Pergamon Press, Oxford 1967.
10 M. J. Berridge, in: Advances in Cyclic Nucleotide Research, vol. 6, p. 1. Ed. P. Greengard and G. A. Robison. Raven Press, New York 1975.
11 W. W. Douglas, Biochem. Soc. Symp. *39*, 1 (1974).
12 B. Katz, The Release of Neural Transmitter Substances. Liverpool University Press, Liverpool 1969.
13 P. F. Baker, Prog. Biophys. mol. Biol. *24*, 177 (1972).
14 E. Alnaes and R. Rahamimoff, J. Physiol. (Lond.) *248*, 285 (1975).
15 H. E. Statham and C. J. Duncan, Life Sci. *17*, 1401 (1975).
16 H. E. Statham and C. J. Duncan, Eur. J. Pharmac. *39*, 143 (1976).
17 H. E. Statham and C. J. Duncan, J. Physiol. (Lond.) *268*, in press (1977).
18 R. I. Birks and M. W. Cohen, Proc. R. Soc. B. *170*, 381 (1968).
19 R. I. Birks and M. W. Cohen, Proc. R. Soc. B. *170*, 401 (1968).
20 P. F. Baker and A. C. Crawford, J. Physiol. (Lond.) *247*, 209 (1975).
21 K. Onodera and K. Yamakawa, Jap. J. Physiol. *16*, 541 (1966).
22 P. N. Benoit, M. L. Audibert-Benoit and M. Peyrot, Archo. ital. Biol. *3*, 323 (1973).
23 J. J. Carmody and P. W. Gage, Brain Res. *50*, 476 (1973).
24 D. D. Branisteanu and R. L. Volle, J. Pharmac. exp. Ther. *194*, 362 (1975).
25 A. C. Crawford, J. Physiol. (Lond.) *246*, 109 (1975).
26 M. P. Blaustein and C. J. Oborn, J. Physiol. (Lond.) *247*, 657 (1975).
27 B. Rose and W. R. Loewenstein, J. Memb. Biol. *5*, 20 (1971).
28 D. A. Lowe, B. P. Richardson, P. Taylor and P. Donatsch, Nature (Lond.) *260*, 337 (1976).
29 M. Crompton, M. Capano and E. Carafoli, Eur. J. Biochem. *69*, 453 (1976).

Sucrose activation of mitosis in lemon fruit explants (Citrus limon L.)

H. A. Kordan[1]

Department of Plant Biology, University of Birmingham B15 2TT (England), 22 December 1976

Summary. Lemon fruit explants manifest mitotic activity when incubated on a single component nutrient medium consisting solely of an aqueous sucrose solution.

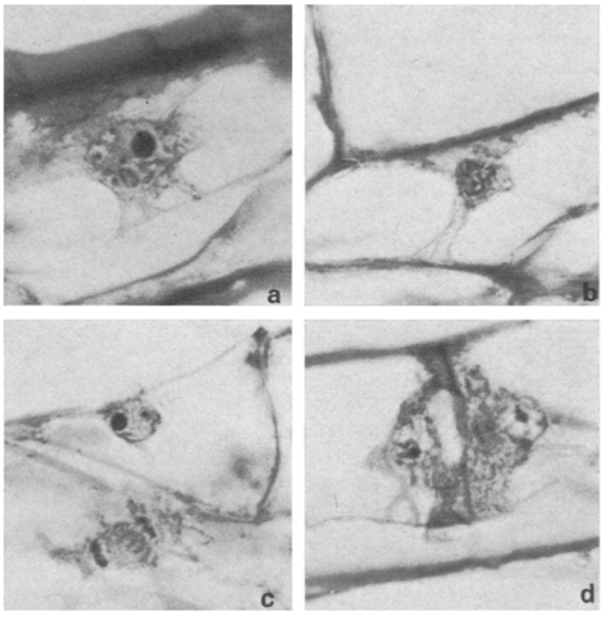
Excised juice vesicle stalks from mature lemon fruits were observed to be capable of manifesting mitotic activity when incubated on a calcium-potassium-sucrose-agar nutrient medium[2]. Although this nutrient medium was far simpler in composition than any used previously for bringing about mitotic activity in lemon fruit explants, the possibility could not be ruled out that Oxoid Agar No. 3 may have supplied the tissue explants with other mineral nutrients known to be essential for plant growth[2]. The manifestation of mitotic activity in lemon fruit explants incubated on sucrose alone on an all-glass physical substratum is reported here.

Materials and methods. Entire juice vesicles (sac plus stalk) were removed aseptically from firm mature yellow lemon fruits with green buttons (Citrus limon L.) as described previously[3] and floated on the surface of sterile glass-distilled water in 'Pyrex' Petri dishes immediately upon removal from the fruit. The stalks were severed from the sacs, the sacs were discarded, and the stalks were transferred to 'Pyrex' Petri dishes lined with Whatman GF/A glass fibre paper saturated with a) sterile glass-distilled water, and b) sterile 4% sucrose solution. The Petri dishes of both treatments were sealed with 'Parafilm' and placed in continuous darkness at 26–27 °C.

After 6 days of incubation, all stalks of both treatments were fixed in Lillie's AAF solution[4], dehydrated with isopropanol and embedded in paraffin wax as described previously[5], and sectioned at 10 μm thickness. The sections were mounted on glass microscope slides without any adhesive, dewaxed in xylene, brought down to water through a graded isopropanol series, and stained with 1% aqueous Safranin-O for 30 min. The Safranin-stained sections were rinsed thoroughly with glass-distilled water, stained with 1% alcoholic Fast-Green FCF for 5 sec, and the Fast-Green staining action stopped by quickly immersing and gently agitating the slide in absolute isopropanol. The sections were thoroughly dehydrated in 2 more changes of absolute isopropanol and mounted in Euparal.

Results and discussion. There was no evidence of mitotic activity in any of the explants incubated on glass-distilled water (treatment a) whereas mitotic figures repre-

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).