level. Reperfusion resulted in a further depletion of tissue GSH content concomitant with a significant increase of GSSG ($p < 0.01$), resulting in a severe reduction of the GSH/GSSG ratio. The table also shows that ischemia and reperfusion did not alter the glutathione reductase and glutathione peroxidase activities measured in the heart homogenates.

Discussion. This study shows that in the isolated rabbit heart ischemia and reperfusion alter the glutathione status.

These alterations cannot be ascribed to a modification of glutathione reductase or glutathione peroxidase activities as both values remained relatively constant under our experimental conditions. They could be the result of the reperfusion-induced lesion of the cell membrane leading to a breakdown of the permeability barrier to molecules such as CPK and glutathione (fig., C).

However, there are three observations that support the idea that the reperfusion-induced glutathione release is not the only cause of the cellular changes which we have observed: 1) tissue GSH content was reduced after ischemia, when the release of glutathione was low, probably as result of the severe reduction of coronary flow; 2) the net amount of glutathione released during reperfusion does not quantitatively account for the cellular reduction of GSH; 3) the finding of a significantly increased GSSG level after reperfusion suggests an enhanced cellular oxidation of GSH into GSSG.

Therefore, it is likely that the cellular alterations of the glutathione status which we observed are the results not only of the glutathione leakage, but also of an enhanced metabolic utilization of GSH, mainly via glutathione peroxidase activity as demonstrated by the increased tissue GSSG[17].

A low value of the GSH/GSSG ratio is deleterious for cell function[18], and in muscle preparations it may increase lipid membrane peroxidation[19] and impair contractile activity[20]. Recently it has been proposed that intracellular thiols, and particularly GSH, may prevent alteration of Ca^{2+} homeostasis reducing lipid peroxidation of the membrane and protecting thiol groups critical for several enzymatic activities, as for example microsomal Ca^{2+}-ATPase[21].

In our experimental conditions, the severe tissue GSH depletion was coincident with an increase of diastolic pressure, an abnormality linked to an enhanced cytosolic Ca^{2+} concentration[12]. This suggests a possible role for glutathione in the determination of the functional damage occurring in post-ischemic reperfusion.

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2 To whom reprint requests should be addressed.
3 Sies, H., Gerstenecker, L., Summer, K.H., Menzel, H., and Flohé, L., in: Glutathione, p.261. Eds L. Flohé, H.Ch. Benöhr, H. Sies, H.D. Waller and A. Wendel. Georg Thieme Publishers, Stuttgart 1974.
4 Wendell, P.L., Biochem. J. *117* (1970) 661.
5 Harish, G., and Mohmound, M.F., Hoppe-Seylers Z. physiol. Chem. *361* (1980) 1859.
6 Harrap, K.R., Jackson, R.C., Riches, P.G., Smith, C.A., and Hill, B.T., Biochim. biophys. Acta *310* (1983) 104.
7 Griffith, O.W., and Meister, A., Proc. natl. Acad. Sci. USA *76* (1979) 56P6.
8 Doroshow, J.H., Locker, G.Y., Baldinger, J., and Myers, C.E., Res. Commun. chem. Path. Pharmac. *26* (1979) 285.
9 Guarnieri, C., Flamigni, F., and Rossoni Caldarera, C., Biochem. biophys. Res. Commun. *89* (1979) 678.
10 Guarnieri, C., Flamigni, F., and Caldarera, C.M., J. molec. cell. Cardiol. *12* (1980) 797.
11 Nayler, W.G., Yepez, C.E., Fassold, E., and Ferrari, R., Am. J. Cardiol. *42* (1978) 217.
12 Ferrari, R., Di Lisa, F., Raddino, R., and Visioli, O., J. molec. cell. Cardiol. *14* (1982) 737.
13 Tietze, F., Analyt. Biochem. *27* (1969) 502.
14 Bergmeyer, H.U., ed., Methods of Enzymatic Analysis, vol.1, p.465. Academic Press, New York 1974.
15 Grankvist, K., Marklung, S.L., and Täljedal, I.B., Biochem. J. *199* (1981) 393.
16 Bradford, M.M., Analyt. Biochem. *72* (1976) 246.
17 Sies, H., Gerstenecker, C., Menzel, H., and Flohé, L., FEBS Lett. *27* (1972) 171.
18 Högberg, J., and Kristoferson, A., Eur. J. Biochem. *74* (1977) 77.
19 Doroshow, J.H., Locker, G.Y., Baldinger, J., and Myers, C.E., Res. Commun. chem. Path. Pharmac. *26* (1979) 285.
20 Kosower, E.M., Experientia *26* (1970) 760.
21 Jones, D.P., Thor, H., Smith, M.T., Jewell, S.A., and Orrenius, S., J. biol. Chem. *258* (1983) 6390.

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Longitudinal continuity of the subrhabdomeric cisternae in the photoreceptors of the compound eye of the drone, *Apis mellifera*[1]

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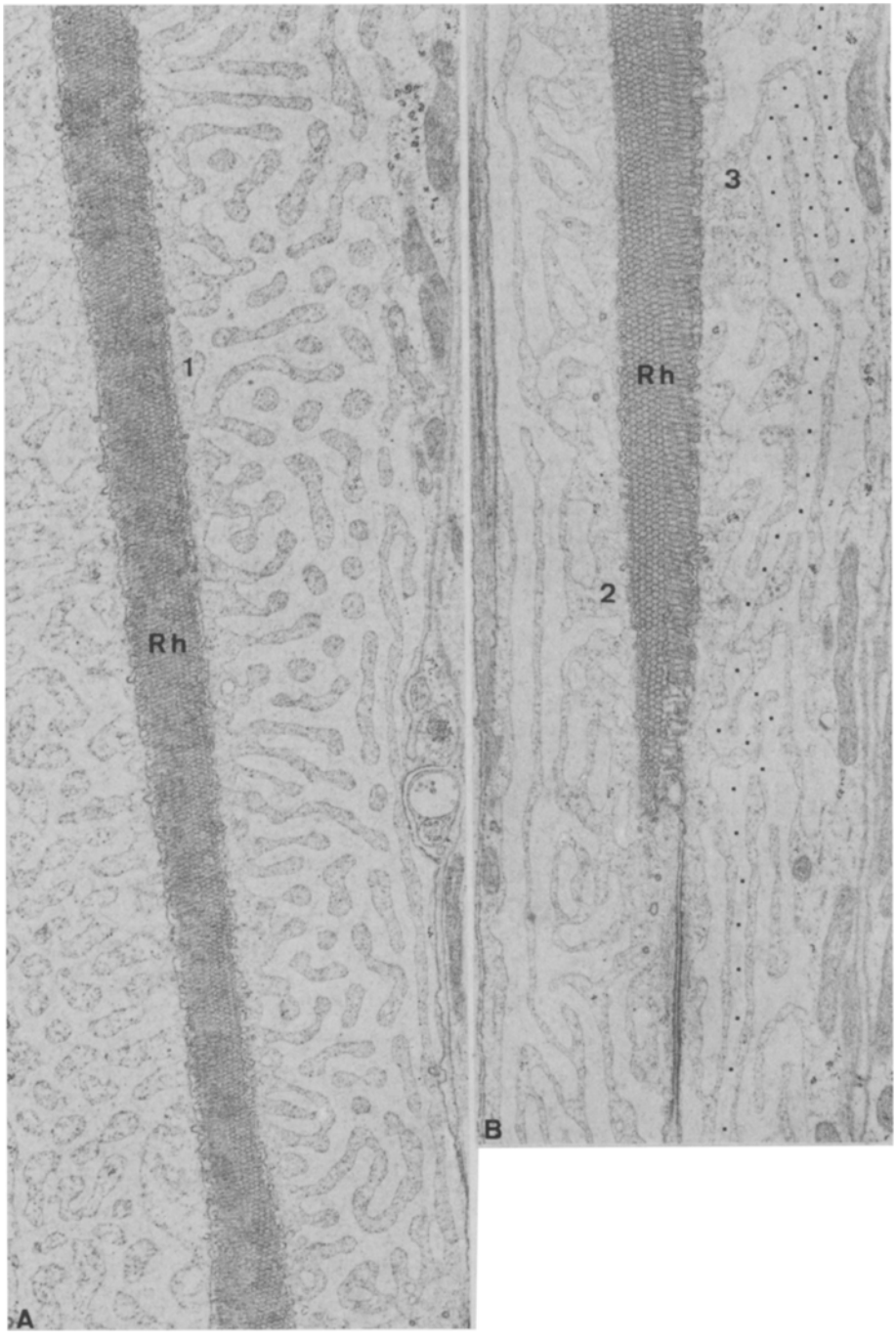
Summary. It is shown that the subrhabdomeric cisternae of the honey bee drone photoreceptor cell constitute a single structure with a continuous lumen, that extends over at least 15 µm and perhaps the whole length of the cell. In this case, the structure of the cisternae might subserve the propagation of light adaptation along the cell.
Key words. Honey bee; *Apis mellifera;* drone; compound eye; photoreceptors; cisternae, subrhabdomeric.

The basic functional unit behind each facet of the drone compound eye is a cluster of six large and three small photoreceptor cells which constitute the retinula, a structure about 400 µm long and 20 µm across[3]. Each cell contributes microvilli to an orderly array, the rhabdom, about 2 µm by 6 µm, which runs down the center of the retinula. In vivo, light passes along the rhabdom, which acts as a light guide, and is absorbed by photopigment in the membranes of the microvilli. Light absorption causes an increase in Na^+ conductance of the cell membrane[4,5] which leads to the electrical response. In addition, there is evidence that light causes an increase in cytosolic free $[Ca^{2+}]$[6,7], in agreement with more extensive evidence from another microvillar photoreceptor cell, that of *Limulus* ventral eye[8,9]. One probable function of this increase in $[Ca^{2+}]$ is to adapt the sensitivity of the photoreceptor to different mean light intensities[6,10].



A) and B) thin sections of the honeybee drone retina cut parallel to the long axis of the photoreceptor cells. The longitudinally cut rhabdom (Rh) is visible in the center of the retinula. To the left and right of each rhabdom is part of a photoreceptor cell: the cytoplasm appears denser than the subrhabdomeric cisternae. In three cells (cytoplasm marked 1,2,3) the lumen runs the entire length of the picture, about 15 µm in A and 12 µm in B. In cell 3 a continuous pathway through the cisternae is indicated by dots. A, × 11,500; B, × 12,000.

As in most species with microvillar photoreceptors, there is, close to the rhabdom, a smooth endoplasmic reticulum, the subrhabdomeric cisternae. Perrelet and Bader[11] showed, by ultrastructural pyroantimonate cytochemistry, that the subrhabdomeric cisternae of the drone contain Ca^{2+} at a concentration much higher than in the cytosol, and they discussed the possibility that at least part of the light-induced increase in free $[Ca^{2+}]$ is due to release from this compartment. In *Limulus* ventral photoreceptor it appears that the light-induced increase in free $[Ca^{2+}]$ does come from an intracellular store, which might be the subrhabdomeric cisternae, since it persists in the absence of external Ca^{2+} (Brown and Blinks[8]), and in the drone, too, physiological experiments argue in favor of an internal source[12]. Further, in another insect, *Calliphora,* the subrhabdomeric cisternae have been shown to accumulate Ca^{2+} in the presence of ATP[13].

In physiological experiments, the rhabdom can be illuminated from the side. When the illumination is confined to a small part (< 20 µm) of its total length, it is found that light adaptation nevertheless spreads over the whole length of the cell[14]. Adaptation is not induced simply by depolarization of the cell membrane[6], and its spread is too rapid to be accounted for by simple diffusion of an intracellular ion[15]. In searching for some other mechanism by which a signal might propagate the length of the cell, we were prompted by the observation of Walz[13] that in *Calliphora* the lumen of the subrhabdomeric cisternae is continuous, at least over short distances. We report that in the drone the subrhabdomeric cisternae appear to form a single

compartment that may run the whole length of the photoreceptor cell.

Methods. Drones were obtained from Mr N. Merin, Chicun Amal, Hadera, Israel, and kept in an unilluminated, but not light-tight, cupboard for up to three weeks, fed by workers who were supplied with sucrose and water. The head was cut off under a table lamp within 5 min of removal from the cupboard, and by slicing parallel to the axes of the retinulae a piece of retina corresponding to about 1 mm square of cornea was obtained. A thread was attached to the cornea with Cyanolit and used to suspend the tissue so that the retinulae remained straight. After fixation for 4 h in glutaraldehyde 3.25%, in a standard cacodylate buffer (75 mM, pH 7.3), the tissue was washed overnight in the buffer. It was postfixed for 1 h in osmium tetroxide 2%, in the buffer, dehydrated in an ethanol series, and embedded in Spurr's resin[16]. Ultrathin sections (600–700 Å) were cut parallel to the long axes of the retinulae with a Reichert-Jung Ultracut and stained first with uranyl acetate 2%, in ethanol 50%, and then with lead citrate according to Reynolds[17]. The sections were examined with a Philips EM 300 operated at 80 kV.

Results and discussion. In most sections the subrhabdomeric cisternae had the fenestrated appearance described previously by Perrelet[3] for the drone and Walz[13] for *Calliphora*. Thirteen photographs were examined of sections cut accurately parallel to the rhabdom, as in the figure. In all of these, a lumen could be traced that was continuous over a distance corresponding to at least 10 µm of rhabdom. In 10 of the 13, the continuity of the lumen extended over the whole field of the photograph. In the 3 cases where a barrier was observed, it was not associated with any obvious specialized structure and the simplest explanation is that the lumen passed out of the plane of the section. In figure B the continuity of the lumen is indicated over a distance of 12 µm, and we suggest that it may extend over the whole length of the photoreceptor cell. From figures A and B, and from the earlier work of Perrelet[3], it appears that the cisternae form an elongated cylinder traversed longitudinally by tubes of cytoplasm. These tubes have side branches that join the bulk of the cytoplasm, particularly on the side close to the rhabdom. Thus, the cisternal compartment has a very high surface-to-volume ratio, a feature which would facilitate rapid exchanges between the interior of the cisternae and the cytoplasm.

The sarcoplasmic reticulum of striated muscle is another endoplasmic reticulum with elongated elements and these elements are presumably capable of propagating a signal from the T system to the center of the sarcomere, a distance of about 1 µm (see, e.g., Endo[18]). The distance over which light adaptation can spread in the drone photoreceptor is at least 200 times greater, but the present results raise the possibility that the subrhabdomeric cisternae have a feature that might be useful for propagating a signal, namely continuity of the lumen.

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3 Perelet, A., Z. Zellforsch. mikrosk. Anat. *108* (1970) 530.
4 Fulpius, B., and Baumann, F., J. gen. Physiol. *53* (1969) 541.
5 Coles, J.A., and Orkand, R.K., J. Physiol., Lond. *332* (1982) 16P.
6 Bader, C.R., Baumann, F., and Bertrand, B., J. gen. Physiol. *67* (1976) 475.
7 Levy, S., M. Sc. thesis, Geneva University, 1979.
8 Brown, J.E., and Blinks, J.R., J. gen. Physiol. *64* (1974) 643.
9 Levy, S., Ph.D. thesis, Boston University, 1983.
10 Brown, J.E., and Lisman, J.E., Nature, Lond. *258* (1975) 252.
11 Perrelet, A., and Bader, C.R., J. Ultrastruct. Res. *63* (1978) 237.
12 Raggenbass, M., J. Physiol., Lond. *344* (1983) 525.
13 Walz, B., J. Ultrastruct. Res. *81* (1982) 240.
14 Bader, C.R., Baumann, F., Bertrand, B., Carreras, J., and Fuortes, G., Vision Res. *22* (1982) 311.
15 Kolatte, E., and Poitry, S., Experientia *39* (1983) 636.
16 Spurr, A.R., J. Ultrastruct. Res. *26* (1969) 31.
17 Reynolds, E.S., J. Cell Biol. *17* (1963) 208.
18 Endo, M., Physiol. Rev. *57* (1977) 71.

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Effect of mercuric acetate on mobilization of N and P during germination and seedling growth of *Cicer arietinum*

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Summay. Mercuric acetate, at 5.0×10^{-5} M, stimulates the mobilization of total nitrogen and phosphate reserves from cotyledons during seedling growth in *Cicer arietinum* cv H208 whereas it suppresses the same process at 2.5×10^{-4} M.

Key words. Cicer arietinum; mercuric acetate; seedling growth; germination.

Mercury, one of the most toxic heavy metals, is known to be accumulated by the vegetation in Hg-polluted areas[2] and to have deleterious biological effects. Severe effects of Hg on plant processes like photosynthesis, respiration and transpiration, etc. have been observed by several workers[3]. In contrast, we found that mercury (as mercuric acetate) at lower concentrations promotes the formation of chlorophylls in etiolated seedlings of cucurbits and germination and seedling development in certain legumes whereas, at higher concentrations, it causes an inhibition in all these parameters[4]. To sort out the basis for the differential effect of Hg concentrations on seedling establishment, the work was extended to investigate the effects of mercuric acetate on mobilization of N and P reserves from cotyledons to the growing axis in *Cicer arietinum*.

Uniform, surface-sterilized seeds of *Cicer arietinum* cv H208 were soaked at 25 ± 2 °C for 24 h in distilled water (control) and 5.0×10^{-5} M and 2.5×10^{-4} M mercuric acetate solutions. Thereafter, 50 seeds were transferred to wet filter papers in Petri dishes for germination in the dark at 25 ± 2 °C. Samples were taken at 5, 7 and 9 days after imbibition. Total nitrogen was estimated by the micokjeldahl method[5], and total phosphate was determined spectrophotometrically at 625 nm using molybdate[6].

Mobilization of total nitrogen and phosphate reserves from cotyledons during development of the seedling is observed to be differentially affected by mercuric acetate concentrations (figs 1 and 2). In the control, total N-content of radicle and epicotyl increases steadily with the age of the seedling, and this