

SEMIFUSED RHABDOM OF THE LADYBIRD BEETLE *COCCINELLA SEPTEMPUNCTATA* LINNAEUS (COLEOPTERA: COCCINELLIDAE)

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The retinular organization of the compound eyes of the ladybird beetle, *Coccinella septempunctata* L., were examined by electron microscopy. The ladybird beetle has two compound eyes without ocelli. Each compound eye is composed of 980 ± 50 ommatidia. The general structure of the compound eyes of *C. septempunctata* L. is similar to those of other insects. Each ommatidium is $150\text{--}200\mu$ in length and $20\text{--}30\mu$ in diameter, and consists of a corneal lens, crystalline cone, pigment cells, eight retinula cells (photoreceptors), and two types of rhabdoms. However, the arrangement of the retinula cells and rhabdoms is unique. The central rhabdom (about 170μ in length) is elongated from the bottom of the crystalline cone to the basement membrane, and is surrounded by the two central retinula cells. The peripheral rhabdom is short (about 10μ in length) in the distal layer only; it encloses the central cells, and is surrounded by the six peripheral retinula cells. The rhabdomic construction of the ommatidium of *C. septempunctata* L. is very different from those found in other insects; it is neither the fused type found in most insects, nor the open type found in dipterans. Instead, it may be regarded as a special "semifused" type of rhabdomic pattern.

Key words: Coleoptera, *Coccinella septempunctata* L., Compound eyes, Semifused rhabdom.

The ladybird beetle, *Coccinella septempunctata* L., is one of the world's most common species of aphidophagous beetles (Nakamuta, 1984; Nakamuta and Saito, 1985). They are predatory in both larval and adult stages, therefore they have become an important insect for the biological control of aphids.

The compound eyes of several species of beetles have been studied, their retinula cells (including centrioles and the ciliary rootlets) have been reported on (Home, 1972, 1976; Holmes, 1975). Other beetles-such as the toad bug (Burton and Stockhammer, 1969), stag beetle (Gokan *et al.*, 1986a), dung

beetle (Meyer-Rochow, 1978; Gokan, 1989a, 1989b; 1990), skin beetle (Gokan and Meyer-Rochow, 1987), dynastine beetle (Gokan *et al.*, 1986b), and asiatic garden beetle (Meyer-Rochow and Gokan, 1987) have also been examined concerning the fine structure of their compound eyes. Most of these beetles have fused rhabdoms; however, the asiatic garden beetle and stag beetle have flower-like rhabdoms which overlap with neighboring cell rhabdoms. Also, the toad bug has an open rhabdom. However, there is insufficient information about the structure of compound eyes in Coleoptera. For the present study, the retinular organization of the compound eyes of the ladybird beetle

was observed using an electron microscope, then compared with other coleopterans. The unique rhabdomeric pattern of the ladybird beetle was observed as a "semifused" type.

MATERIALS AND METHODS

Male beetles were used throughout this research. Samples were immobilized by chilling, after which the heads were dissected and fixed in 2.5% glutaraldehyde buffer with 0.1 M cacodylate (pH 7.3) at 4°C. Samples were then dehydrated, fixed, embedded and observed by methods described by Wu *et al.* (1985).

RESULTS

Gross structure

Ladybird beetles have compound eyes without ocelli on each side of the head. Each eye is an oval hemisphere with an anterior-to-posterior orientation, and consists of 980 ± 50 ommatidia (Fig. 1A). Each ommatidium has a hexagonal or several modified hexagonal lenses about 30μ in diameter on its surface (Fig. 1B). The surface of each lens is flat, with a slight depression where one lens comes in contact with its adjacent lens. As in some insects, the corneal nipple array is absent from ladybird beetle eyes. The short (about 5μ) interfacet hairs at the lens junctions are found approximately every 3-4 lenses (Figs. 1B and 1C).

Longitudinal section

The arrangement of the retinula cells and the two types of rhabdoms found in the ommatidia are shown in Fig. 4. Each ommatidium contains a corneal lens, a crystalline cone, pigment cells, and receptor layers (Figs. 1D and 4). The corneal lenses of the central ommatidia are about 60μ in thickness; they are biconvex (protruding inward)

and have many laminations. The crystalline cone beneath the corneal lens consists of four Semper's cells, and is enclosed in a pair of primary pigment cells. The nuclei of the primary pigment cells are at the base of the crystalline cone. The secondary pigment cells surround both the primary pigment and retinula cells. The basal pigment cells are connected to the basement membrane, and their nuclei are situated in the innermost part of the cells. The receptor layer is $150\text{--}170\mu$ in length, and is elongated from the crystalline cone to the basement membrane. Near the basement membrane, all of the retinula cells are attenuated and transformed into axons, which penetrate the basement membrane and run to the optic lobes.

The ommatidium consists of two independent rhabdoms; the longer rhabdom, located in the central area, runs along the ommatidial axis through the entire receptor layer. We named it the central rhabdom (Fig. 1D). The other, in the peripheral area, is short with a cylindrical form; it can only be found in the distal part. We named these the peripheral rhabdoms (Figs. 1D, 2 and 4). These rhabdoms have a length of only 10μ , measured from below the base of the crystalline cone. The nuclei of the peripheral retinula cells are situated in the distal layer where the peripheral rhabdoms appear. The nuclei of the central retinula cells lie in the middle layer where the peripheral rhabdoms disappear entirely (Fig. 4).

Cross section

The arrangement of the eight retinula cells and the two separated rhabdoms in a single ommatidium is unique. In the distal part of an ommatidium, the two central retinula cells are thin (about 3μ in diameter), whereas the six peripheral cells share similar diameters (about 4μ). However, the six cells become thinner as they approach to the middle and proximal layers; the thinnest is only about 1μ in diameter. On the other

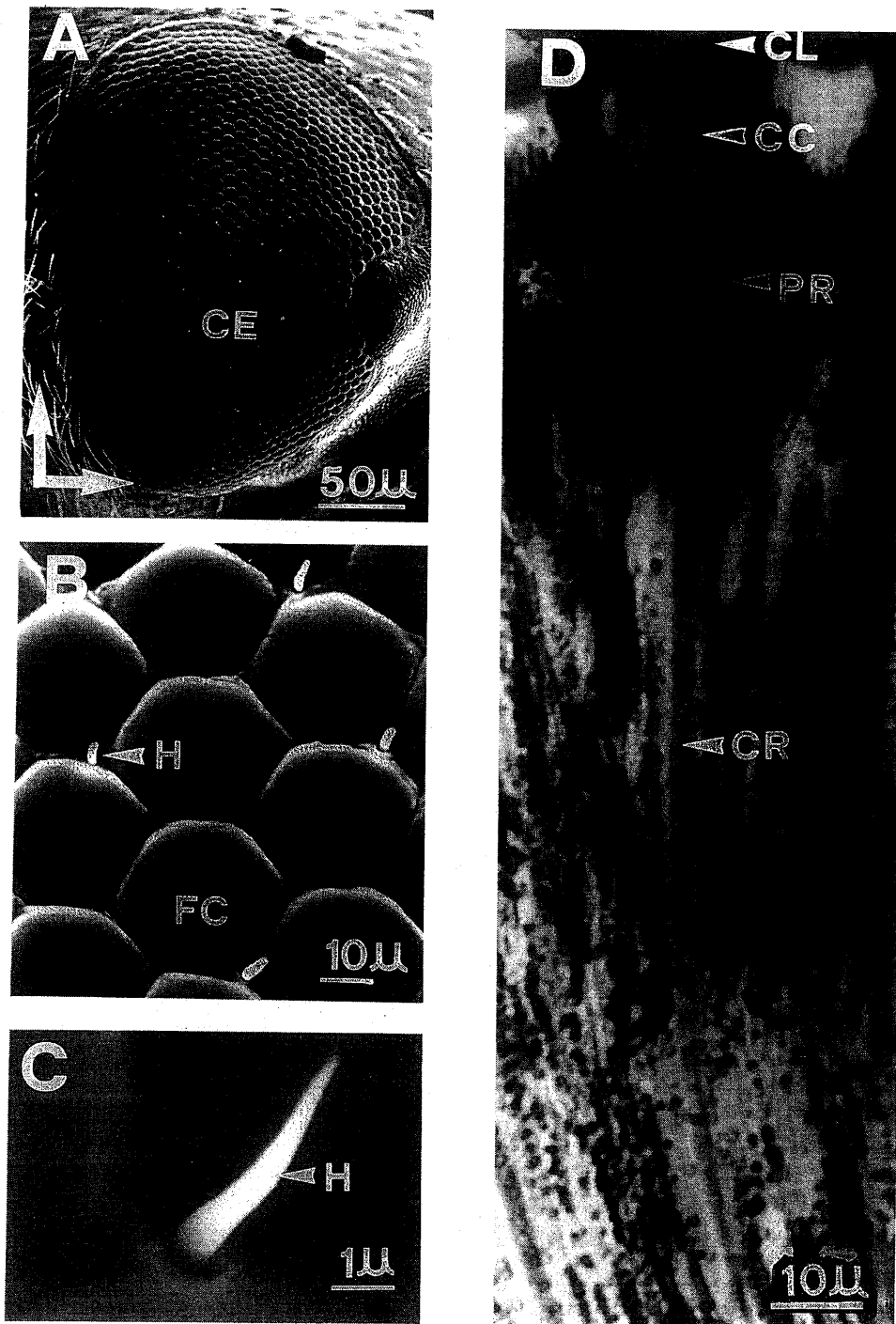


Fig. 1. A. The right eye of *Coccinella septempunctata* L. Arrows show dorsal (up) and anterior directions.
 B. Low magnification photo of several facets and interfacet hairs.
 C. High magnification photo of an interfacet hair.
 D. A longitudinal section from the ommatidia.
 CC: crystalline cone; CE: compound eye; CL: corneal lens;
 CR: central rhabdom; FC: facet; H: interfacet hair;
 PR: peripheral rhabdom.

hand, the two central retinula cells have thicker diameters of about 5μ in the middle layer. The two types of retinula cells show some different cytoplasmic features from layer to layer, especially in the distribution of pigment granules. The central cells usually have few pigment granules in the distal layer, but numerous granules in the middle and innermost layers. The peripheral cells contain many spherical pigment granules $0.5\text{--}0.7\mu$ in diameter in the distal region (where the peripheral rhabdom is found), but are without granules in the middle and innermost layers.

Of course, both types of retinula cells contain various cytoplasmic organelles such as mitochondria, microtubules, endoplasmic

reticula, ribosomes, Golgi apparatuses, multivesicular bodies, and centrioles which are found in other beetles (Home, 1972, 1976; Holmes, 1975; Meyer-Rochow, 1978; Meyer-Rochow and Gokan, 1987; Gokan, 1989a, 1989b, 1990; Gokan and Meyer-Rochow, 1987; Gokan *et al.*, 1986a, 1986b).

The two central retinula cells are connected to the central rhabdoms, whereas the six peripheral cells are connected to the hexagonal peripheral rhabdoms (Figs. 2, 3). The central rhabdom has a cylindrical form about 2.5μ in diameter; it is a construction of the opposing rhabdomeres of the two central retinula cells. The peripheral rhabdom is composed of the rhabdomeres of the six peripheral retinula cells, and was not found to

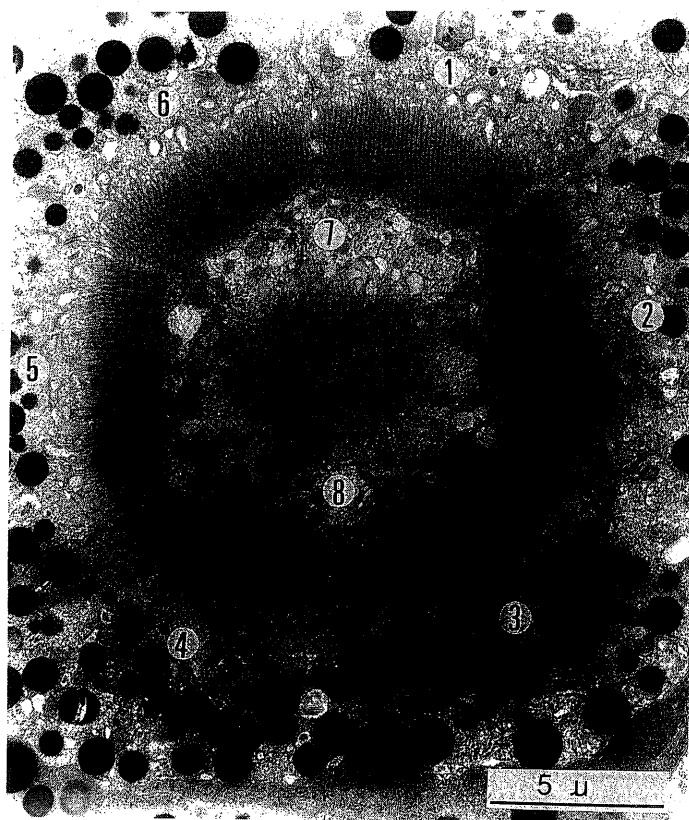


Fig. 2. A cross section through the distal layer of the ommatidium. The peripheral rhabdom is hexagonal, and consists of the six peripheral retinula cells (Nos. 1 to 6), whereas the central rhabdom is triangular and formed by the two central retinula cells (Nos. 7 and 8).

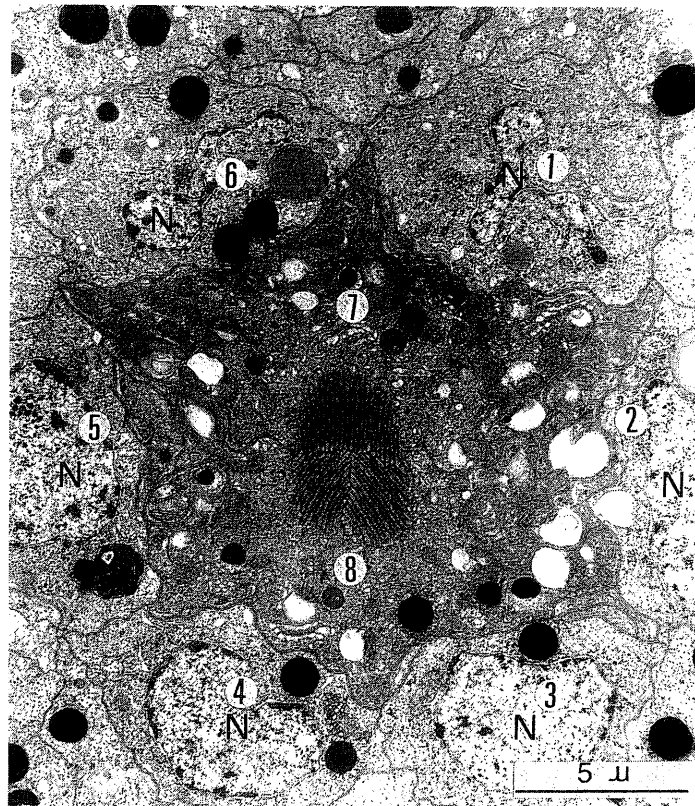


Fig. 3. A cross section through the nuclear level of the six peripheral retinula cells. The peripheral rhabdom disappears completely, and the central rhabdom appears only at this level. N: nucleus.

be connected to the central rhabdom. The peripheral rhabdoms are hexagon-shaped, and completely enclose the two central retinula cells. The distal tips of both the central and peripheral rhabdoms are connected to the crystalline cone base. Both rhabdoms are composed of many microvilli, and there is little difference between the central and peripheral rhabdoms in the rhabdomeric microvilli. The microvilli of each rhabdomere are regularly arranged, but the microvilli axes are not all parallel; they are arranged in three directions in the central rhabdom, and two the peripheral rhabdom. In other words, the peripheral rhabdoms are approximately perpendicular. The central rhabdom is divided into three parts, of which two belong to the No. 8 central cell, and one belongs to the No. 7 central cell (Figs. 2-4).

Rhabdom organization is distinctly different between the distal and proximal layers of an ommatidium. Therefore, rhabdom occupation ratios (ROR) were measured for both the proximal and distal layers. The ROR of the distal level was measured at 20.85%, while it was only 3.5% for the proximal level.

DISCUSSION

Rhabdomeric patterns in the ommatidia of insect compound eyes have been divided into two types: fused and open (Carlson and Chi, 1979). The former is extensively found in numerous species of insects; examples include the cockroach (Butler, 1971), worker bee (Gribakin, 1972), ant (Menzel, 1972),

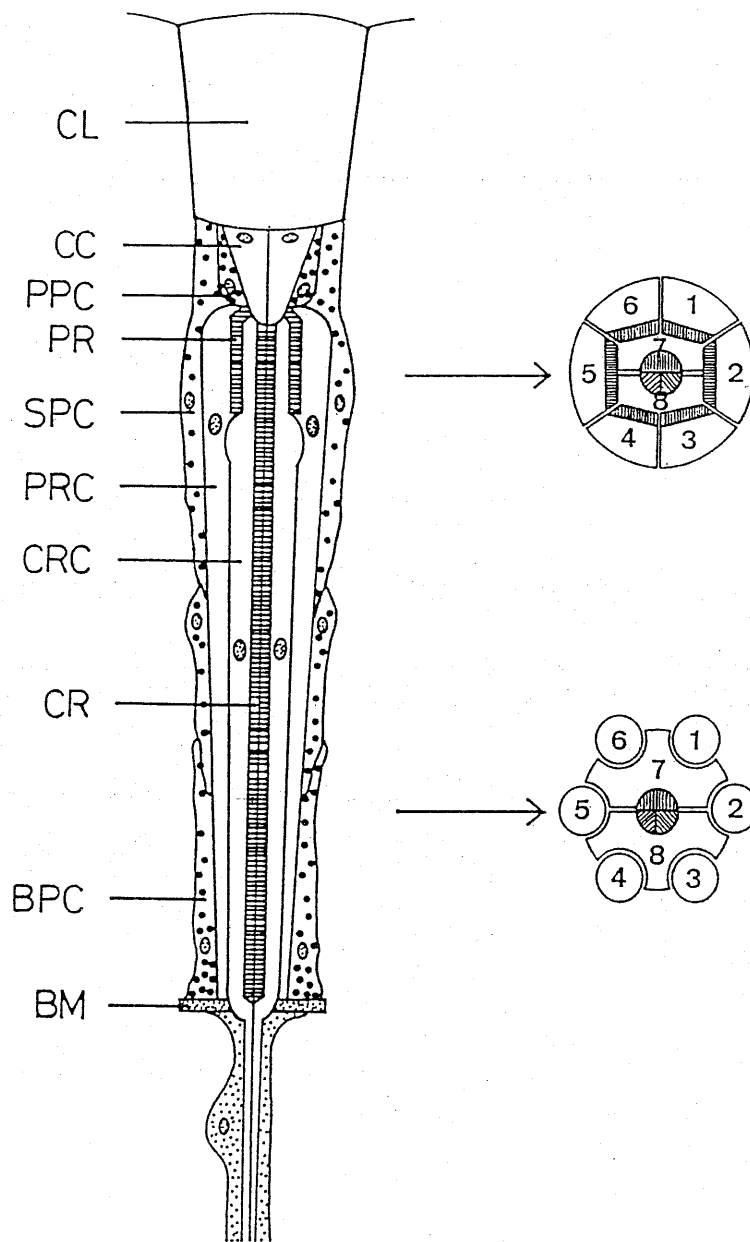


Fig. 4. Longitudinal and cross section diagrams of a typical ommatidium of the ladybird beetle showing the relative positions of the retinula cells and rhabdoms. Striations indicate the orientation of the rhabdomeric microvilli. Numerals indicate the retinula cells.

BM: basement membrane; BPC: basal pigment cell;
 CC: crystalline cone; CL: corneal lens;
 CR: central rhabdom; CRC: central retinula cell;
 PPC: primary pigment cell; PR: peripheral rhabdom;
 PRC: peripheral retinula cell; SPC: secondary pigment cell.

Galloisiana (Gokan *et al.*, 1982), dung beetle (Meyer-Rochow, 1978; Gokan, 1989a, 1989b, 1990), garden beetle (Meyer-Rochow and Gokan, 1987) and skin beetle (Gokan and Meyer-Rochow, 1987). The second type is found in insects such as the mosquito (Brammer, 1970), toad bug (Burton and Stockhammer, 1969) and oriental fruit fly (Wu *et al.*, 1985). In this study, we found that the compound eye of *C. septempunctata* L. has two kinds of rhabdoms. The six peripheral rhabdomeres are situated only in the distal part of the ommatidia, and are connected so as to form a cylindrical shape; they separate the peripheral retinula cells from the central retinula cells. The two central rhabdomeres are fused, and extend from the distal to the innermost part. They are separated from the six peripheral rhabdomeres by the two central retinula cells. Such a unique arrangement shows that the rhabdoms of *C. septempunctata* L. are neither the fused nor open type.

Behavioral observations have shown that *C. septempunctata* L. actively searches for the prey only during daylight (Nakamuta, 1984; Nakamuta and Saito, 1985). We found that the compound eyes of *C. septempunctata* L. are more sensitive to white light than other beetles, and that it may have three kinds of color receptors: UV, blue, and green (Lin and Wu, 1992). Nakamuta (1984) reported that in daylight conditions, adult *C. septempunctata* L. responded to prey at a distance of 7 mm, but in nighttime conditions, they did not respond to prey even at 2 mm distance. The reasons for *C. septempunctata* L. having color receptors and being more sensitive to photoreception than other beetles (Lin and Wu, 1992), yet showing weak visual perception of prey under light conditions are unclear.

Several studies have suggested that rhabdom occupation ratio (ROR) is useful for indicating the degrees of nocturnal activity in insects (Eguchi and Horikoshi, 1984;

Eguchi and Sasaki, 1985; Meyer-Rochow and Gokan, 1987). The ROR of nocturnal moths is very high (40-80%), and for diurnal moths or butterflies it is very low (1-5%). However, a few species of diurnal moths have shown an intermediate value of about 25%. Within an ommatidium, *C. septempunctata* L. has one long central rhabdom and six short peripheral rhabdoms. In the distal part of the ommatidia, the ROR (20.85%) is an intermediate value. In the proximal part, the ROR (3.5%) is typical for diurnal moths or butterflies. The special arrangement of rhabdoms we observed may be related to behavioral changes in response to light conditions. Whether physiological differences between the central and peripheral rhabdoms in *C. septempunctata* L. exist or not remains to be investigated, perhaps by intracellular recording from a single retinula cell.

In conclusion, from a comparison of the compound eyes of *C. septempunctata* L. and those of other beetles, our results show that the fundamental components of the ommatidia of *C. septempunctata* L. agree with those of other beetles. However, *C. septempunctata* L. possesses compound eyes which differ markedly from other beetles in rhabdomeric organization. Therefore, the rhabdoms of *C. septempunctata* L. may be regarded as a special "semifused" type of rhabdomeric pattern.

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七星瓢蟲複眼的半集中型桿狀體

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本實驗以電子顯微技術，觀察鞘翅目七星瓢蟲(*Coccinella septempunctata* L.)複眼的視網膜結構。七星瓢蟲的成蟲有一對複眼，缺乏單眼。每個複眼由 980 ± 50 個小眼組成。每個小眼之長度約為 $150-200 \mu$ ，最大直徑約為 $20-30 \mu$ ，小眼中均有角膜晶體，晶錐體，色素細胞，桿狀體及八個視細胞。七星瓢蟲複眼的構造和一般昆蟲頗相似，然視細胞和桿狀體的排列十分特殊。中央桿狀體很長，約有 170μ ，從頂部晶錐體延伸到基底膜，是由兩個中央視細胞的桿狀體組成，故整條中央桿狀體被該兩個視細胞包圍住。周圍桿狀體很短，長只約 10μ ，只分佈於頂部晶錐體下方 10μ 範圍內，向內緊貼住中央視細胞，外面由六個周圍視細胞圍住。七星瓢蟲複眼視細胞和桿狀體的排列如此特殊，與一般的昆蟲複眼截然不同。桿狀體排列既不屬於如大多數昆蟲的集中型，也不屬於如東方果實蠅等雙翅目昆蟲的分散型。因此，根據桿狀體的特殊排列方式，或許可將之歸為特殊的“半集中型桿狀體”。

EFFECTS OF TREATMENT WITH ARSENITE ON THE CYTOTOXICITY OF R-RAY-IRRADIATED CHINESE HAMSTER OVARY CELLS AND HUMAN FIBROBLASTS

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H. Huang, S.H. Tyan and H.J. Huang (1991) Effects of treatment with arsenite on the cytotoxicity of r-ray-irradiated Chinese hamster ovary cells and human fibroblasts. Bull. Inst. Zool., Academia Sinica 31(4): 270-275. Pretreatment with arsenite for 12 h enhanced the cytotoxicity of r-ray-irradiated Chinese hamster ovary (CHO-K1) cells and human fibroblasts (HF). However, treatment with arsenite increased cellular glutathione in CHO-K1 cells, but not in HF. Post-treatment with arsenite for 12 h had no effect on the r-ray-irradiated CHO-K1 cells, and only slightly enhanced the cytotoxicity of r-ray-irradiated HF. These results are similar to the effects of pre- and post-treatment with arsenite on the cytotoxicity of bleomycin - a radiomimetic drug which also breaks cellular DNA by means of a free radical mechanism and induces both single-strand and double-strand DNA breaks.

Key words: Cytotoxicity, Radiation, Arsenite

For some time arsenic - a human carcinogen - has been known to be genotoxic. Recently arsenite - a trivalent form of arsenic - has been shown to have a modulating effect on the genetic toxicity of many mutagens. Post-treatment with arsenite has been shown to enhance the cytotoxicity of ultraviolet light (Lee *et al.*, 1985; Okui and Fujiwara, 1986), cis-diaminedichloroplatinum (II) (Lee *et al.*, 1986a), methyl methanesulfonate (Lee *et al.*, 1986b), and ethyl methanesulfonate (Jan *et al.*, 1990), but not the cytotoxicity of r-rays (Huang *et al.*, 1989), or bleomycin (Jan *et al.*, 1990). On the other hand, pretreatment with arsenite has been shown to enhance the cytotoxicity of bleomycin (Jan *et al.*, 1990), but not that of methyl methanesulfonate (Lee *et al.*, 1986b). Inasmuch as bleomycin breaks cel-

lular DNA strands through free radicals that mimic the effects of ionizing radiation, and since both bleomycin and ionizing radiation induce both singlestrand and double-strand DNA breaks, experiments were conducted to see if similar modulating effects of arsenite on r-ray-irradiated cells could be observed. This information may help in understanding the types of damage that can be influenced by arsenite.

MATERIALS AND METHODS

Cell cultures

Chemicals for cell cultures were obtained from GIBCO (Grand Island, NY). Sodium arsenite (SA) was purchased from Merck (Darmstadt, F.R.G). Chinese hamster ovary (CHO-K1) cells and human fibroblasts (HF)