PROTEIN SYNTHESIS IN THE VISUAL CELLS

OF THE HONEYBEE DRONE AS STUDIED

WITH ELECTRON MICROSCOPE RADIOAUTOGRAPHY

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ABSTRACT

Protein synthesis was studied in the visual cells of an insect (honeybee drone, *Apis mellifera*) by electron microscope radioautography. After a single injection of tritiated leucine, the radioactivity first appears in the cytoplasm of the visual cell which contains ribosomes. Later, part of this radioactivity migrates to the rhabdome, the visual cell region which is specialized in light absorption. A maximal concentration of radioactivity is reached there 48 hr after the injection of leucine. This pattern of protein synthesis and transport resembles that described in vertebrate visual cells (rods and cones), where newly synthesized proteins have been shown to contribute to the renewal of the photoreceptor membrane.

INTRODUCTION

Until recently, the morphology of invertebrate eyes has been investigated mainly from the point of view of comparative histology. All studies (see review by Eakin, 1972 [6]) have pointed out a cytological differentiation of the visual cell in two main regions, one formed by numerous microvilli and thought to be the site of photoreception, the other, the cell body, containing the usual cytoplasmic organelles. In the cytoplasm of the visual cells, investigators have also noticed an abundance of protein synthesizing organelles (ribosomes either bound to membranes of the endoplasmic reticulum or free); it was therefore assumed that protein synthesis in visual cells is concerned with the renewal of the photopigment. So far, protein synthesis in the visual systems of invertebrates has been studied in two arthropods, the horseshoe crab *Limulus polyphemus* (2) and the honeybee drone *Apis mellifera* (11). In both cases, experiments have shown that the turnover of proteins in the retina after the administration of a labeled amino acid is influenced by light or dark adaptation. Moreover,

radioautography in the horseshoe crab (2) suggested that more labeling is present in the microvilli in a dark adapted animal than in a light adapted one. The precise ultrastructural localization of protein synthesis in visual cells, however, remained largely unknown and the purpose of this study was to examine, with electron microscope radioautography, the fate of radioactivity in the visual cells of the honeybee drone after a single injection of tritiated leucine.

MATERIALS AND METHODS

Adult honeybee drones[1] weighing approximately 200 mg were injected in the dorsal aorta with 80 μCi of tritiated leucine (4–5 ^{3}H, specific activity

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50 Ci/mM, Schwarz Bio Research Inc., Orangeburg, N. Y.) dissolved in 10 μl of Tris-HCl buffer 0.1 M, pH 7.0.

The injection was performed with the aid of a micropipette tip connected to a piece of polyethylene tube calibrated in microliters. The injected drones were kept in individual boxes containing a supply of live worker bees. The worker bees, who see to the feeding of the drones, were given pollen and a 1:1 cane sugar-water solution. The drones, submitted to normal day-night illumination changes, were sacrificed 1, 2, 10, 24, 48, and 96 hr after the injection and their retinas fixed for 2 hr in a cold 2% osmium tetroxide solution in phosphate buffer (10). After alcohol dehydration and Epon embedding, thick sections of the retinas were produced, deposited on clean, uncoated glass slides, and radioautographed for light microscopy with Ilford L4 emulsion diluted 1:1 (4). The intensity of radioautographic reaction was checked after 2 wk of exposure. Thin sections were then prepared from the successful blocks, deposited on 200-mesh copper grids coated with Formvar and carbon, and covered with a film of Ilford L4 emulsion with the loop technique (4). After an exposure of 6 wk, the emulsion was developed for 5 min in Microdol X (Eastman Kodak Co., Rochester, N. Y.), washed for 30 sec in distilled water, and fixed 5 min in Kodak acid fixer. Grids were stained with alcoholic uranyl acetate followed by lead citrate (14), an examined in a Philips EM 300 electron microscope. Photographs were taken at a fixed magnification of × 5600 which enabled most of the ommatidial surface to be reproduced on a 8½ by 10 cm negative plate. Photographic prints (3X) were used for counting the radioautographic grains associated with the different cell components. In some cases, cell components were also cut out of the prints, their surface area estimated by weighing, and the radioactivity expressed in grains per unit area. In addition, radioautographic experiments were performed with retinas fixed in 4% methanol-free formaldehyde buffered with phosphate (8) in order to assess the possible retention of free tritiated leucine by the osmic fixative (1, 13). At the stage of maximal rhabdomeric labeling, the number of radioautographic grains present in the rhabdome was found to be comparable in osmium- and in formaldehyde-fixed tissue (see Fig. 5). The osmium fixative was preferred since it resulted in a better preservation of the tissue than the use of formaldehyde did. The normal ultrastructure presented in this paper has been obtained from the retinas of injected animals fixed in osmium tetroxide. All animals were light adapted at the time of fixation.

RESULTS

Before presenting the results of the radioautographic experiments, a brief summary of the drone visual cell ultrastructure will be given with special emphasis on the organization of the cell cytoplasm. A detailed description of the drone retina has been published previously (12). The drone retina is composed of several thousand ommatidia, each of which is formed of nine visual or retinular cells (see Fig. 4). Fig. 1 shows the central region of an ommatidium. Parts of five visual cells can be seen surrounding a structure formed by closely packed microvilli, the rhabdome. The microvilli are processes of the visual cells (see Fig. 3) and similar structures in the fly (Calliphora) have been shown to contain the photopigment (9). Opposite the rhabdome, i.e., near the periphery of the ommatidium, the visual cell cytoplasm contains numerous profiles of rough endoplasmic reticulum as well as free polysomes (Fig. 2). The cytoplasmic area situated between the rhabdomeric microvilli and the endoplasmic reticulum is occupied by elon-

FIGURE 1 Central region of an ommatidium showing parts of five retinular cells (RC) surrounding a structure formed by microvilli, the rhabdome (rh). Many rough endoplasmic profiles (rer) as well as free polysomes are present in the cytoplasm of the cells and arrows point to the possible connection between the rough endoplasmic reticulum profiles and the perirhabdomial cisternae (c). Golgi complexes (g) are visible in some cells. Note mitochondria (mi) close to the plasma membrane as well as pigment cells processes (PC) wedged in between the visual cells. Beyond the rhabdome, visual cells are joined by junctional complexes (d). × 24,000.

FIGURE 2 High magnification of the ribosomes in the peripheral cytoplasm. Ribosomes appear either associated with endoplasmic reticulum membranes (single arrow) or as free polysomes (double arrow). × 32,000.

FIGURE 3 High magnification of the base of the rhabdomeric microvilli (mv) showing their close relationship (arrows) with the perirhabdomial cisternae (c). The cisternae contain a material of appreciable electron density, higher than that of the cytoplasmic matrix of the visual cells (RC). × 40,000.
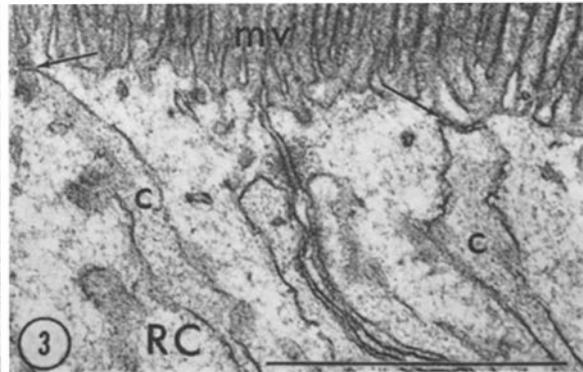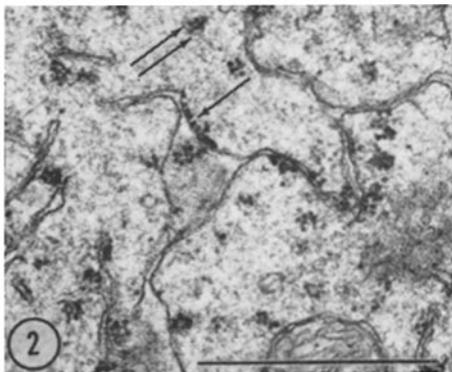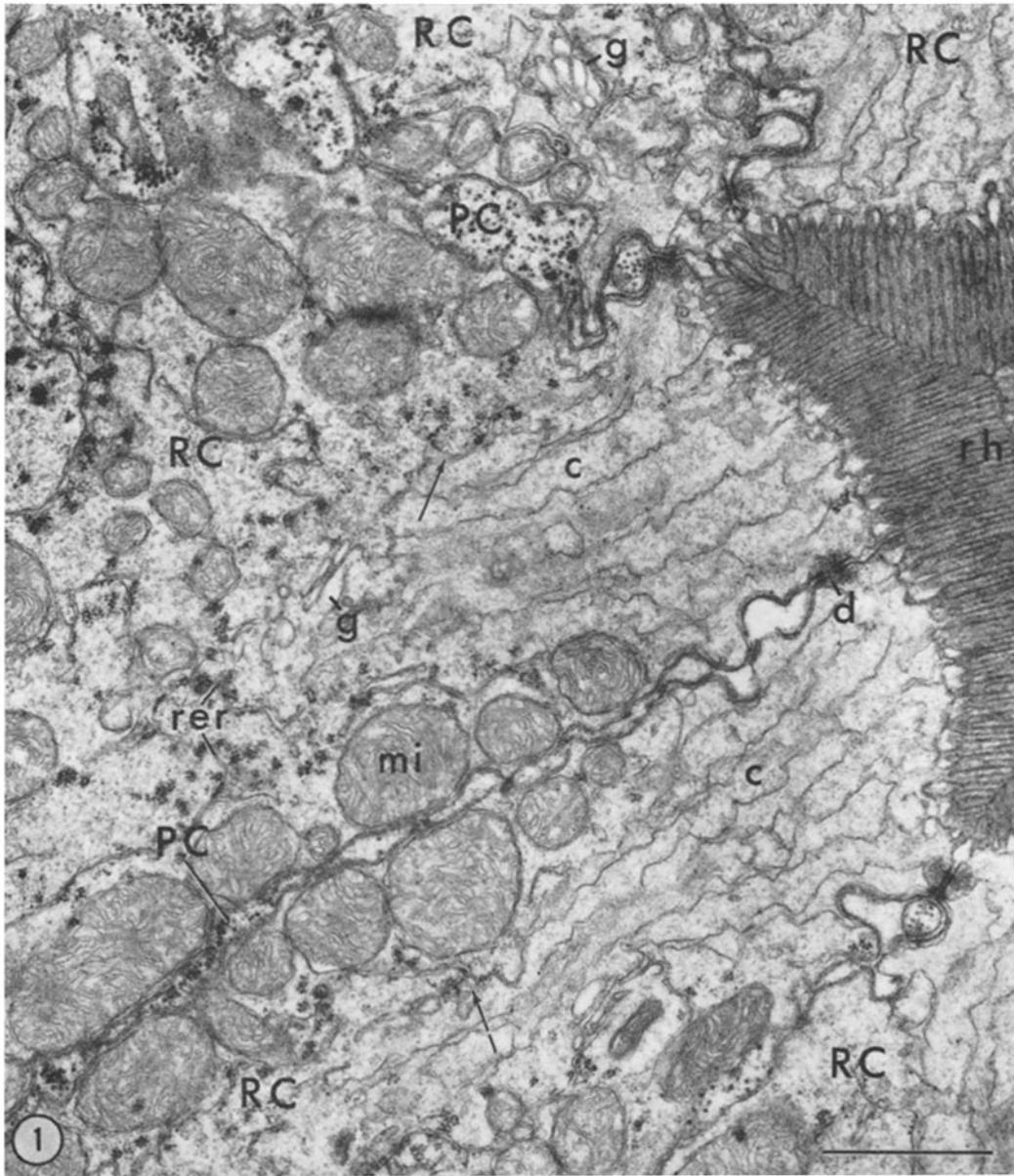
gated, smooth-surfaced membranous profiles. These profiles, the perirhabdomial cisternae, terminate very close to the base of the rhabdomeric microvilli (Fig. 3); however, an opening of the cisternae in the intervillous space could not be observed. On the contrary, the cytoplasmic extremity of the perirhabdomial cisternae seems to be continuous with rough endoplasmic profiles, and a Golgi complex can be seen where the cisternae merge with the endoplasmic reticulum. From the preceding description, it can be concluded that the drone visual cell is divided into three main components: (a) The peripheral cytoplasm containing ribosomes, (b) the perirhabdomial region with smooth surfaced cisternae, and (c) the rhabdomeric microvilli.

48 hr after the injection of a single dose of tritiated leucine in the hemolymph of a drone, radioactivity can be found in all three components of the visual cells (Fig. 5). In this light radioauto-

graph, the labeling suffices to reveal the outline of each ommatidium without additional staining of the preparation being required. The heaviest radioautographic reaction is found in the center of the ommatidia, where the rhabdomeric microvilli are situated.

High resolution radioautographs made at different intervals of time after the injection of the tritiated leucine show that the radioactivity present in the rhabdome seems to originate in the cytoplasm of the visual cells. 1 hr after the administration of leucine (Fig. 6), the radioautographic grains are scattered over the peripheral cytoplasm and the perirhabdomial region. The rhabdome, however, is practically free of radioactivity. At this point, approximately 50% of the radioautographic grains are associated with the peripheral cytoplasm containing the rough endoplasmic reticulun and the free polysomes.

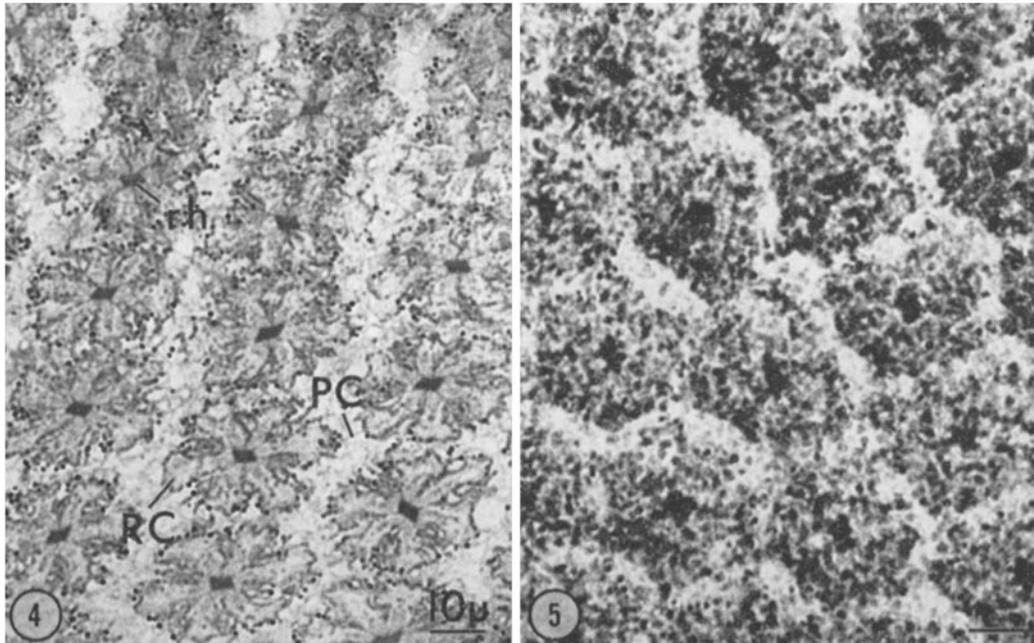48 hr after the injection (Fig. 7), there is still

FIGURE 4   Light micrograph of a drone retina. The retina is composed of numerous ommatidia separated from each other by pigment cells (PC). Individual retinular cells (RC) forming the ommatidia as well as the central rhabdomes (rh) can be seen. Pigment cells contain dark granules not to be confused with radioautographic grains. Thick section stained with methylene blue, without photographic emulsion. × 700.

FIGURE 5   Light microscope radioautography of a drone retina fixed 48 hr after the injection of tritiated leucine. Although this section was not stained, the outlines of the ommatidia are clearly defined by the radioautographic reaction in the cytoplasm of the visual cells. The region of the rhabdomes, in the center of the ommatidia, is heavily labeled with silver grains. Formaldehyde fixation. × 700.
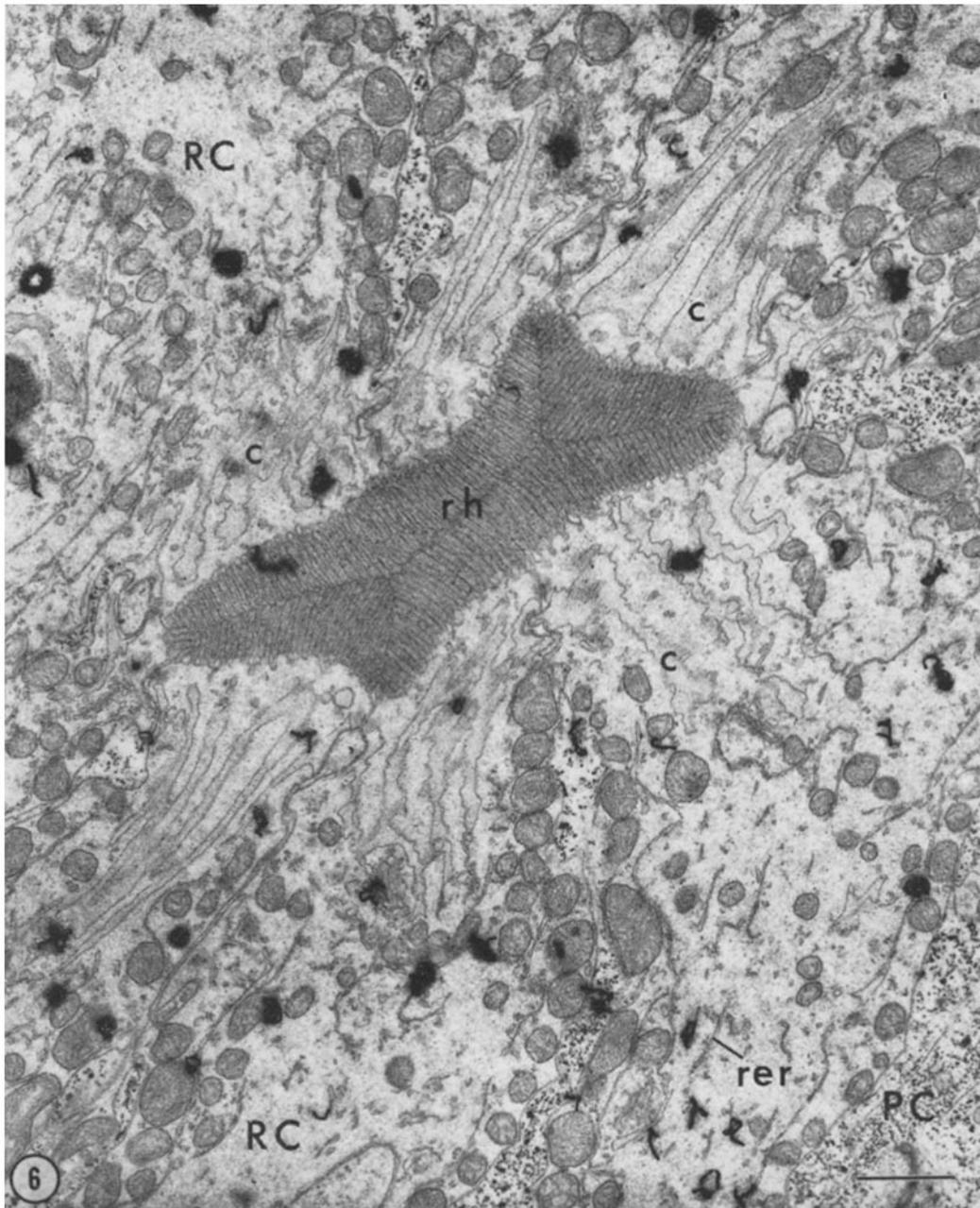
FIGURE 6   Electron microscope radioautography prepared from a retina fixed 1 hr after the injection of leucine. The main cytological features described in Fig. 1 can be seen: The peripheral cytoplasm of the visual cells (RC) rich in rough endoplasmic reticulum profiles (rer) and polysomes, the perirhabdomial cisternae (c), and the rhabdomeric microvilli (rh). The radioautographic grains are mainly associated with the peripheral cytoplasm and with the perirhabdomial region. Very few grains are present over the rhabdome. Pigment cells (PC) are weakly labeled. × 13,000.
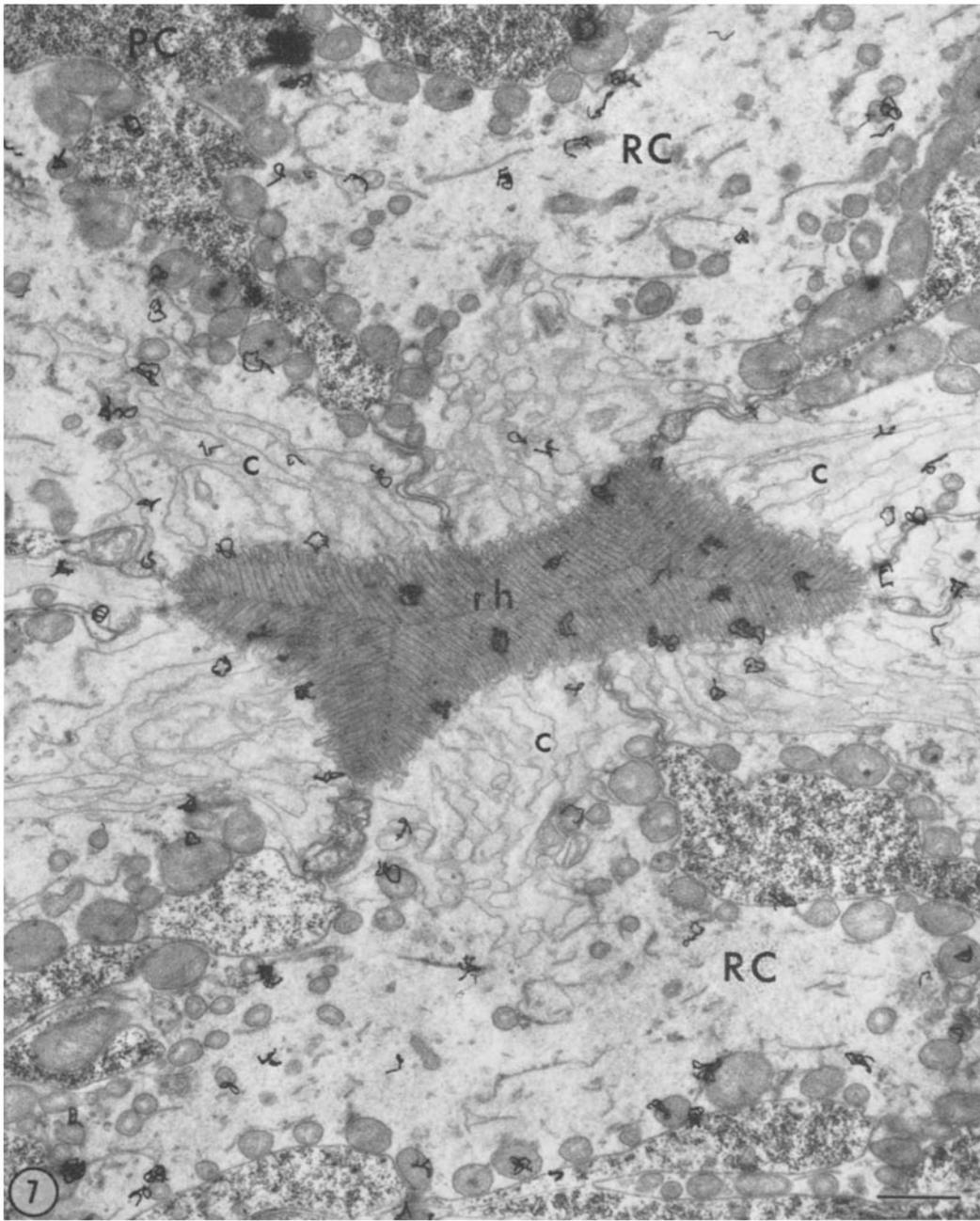
FIGURE 7 Retina fixed 48 hr after the injection of leucine. The labeling of the visual cells (RC) cyto-plasm is slightly reduced compared to that seen in Fig. 6. On the other hand, numerous grains can be seen in the rhabdomeric microvilli (rh). The pigment cells (PC) surrounding the ommatidia are weakly labeled. The two dark spots in the upper part of the picture are granules of screening pigment (ommo-chrome) contained in the pigment cell cytoplasm. × 12,000.

an appreciable amount of labeling in the cell cytoplasm and the perirhabdomial region, but in addition, numerous silver grains are present over the rhabdomeric microvilli of the visual cells (about 20% of the total number of grains). The persistance of cytoplasmic labeling at a high level during the first 48 hr of the experiment might be explained by the upper graph of Fig. 13 which shows
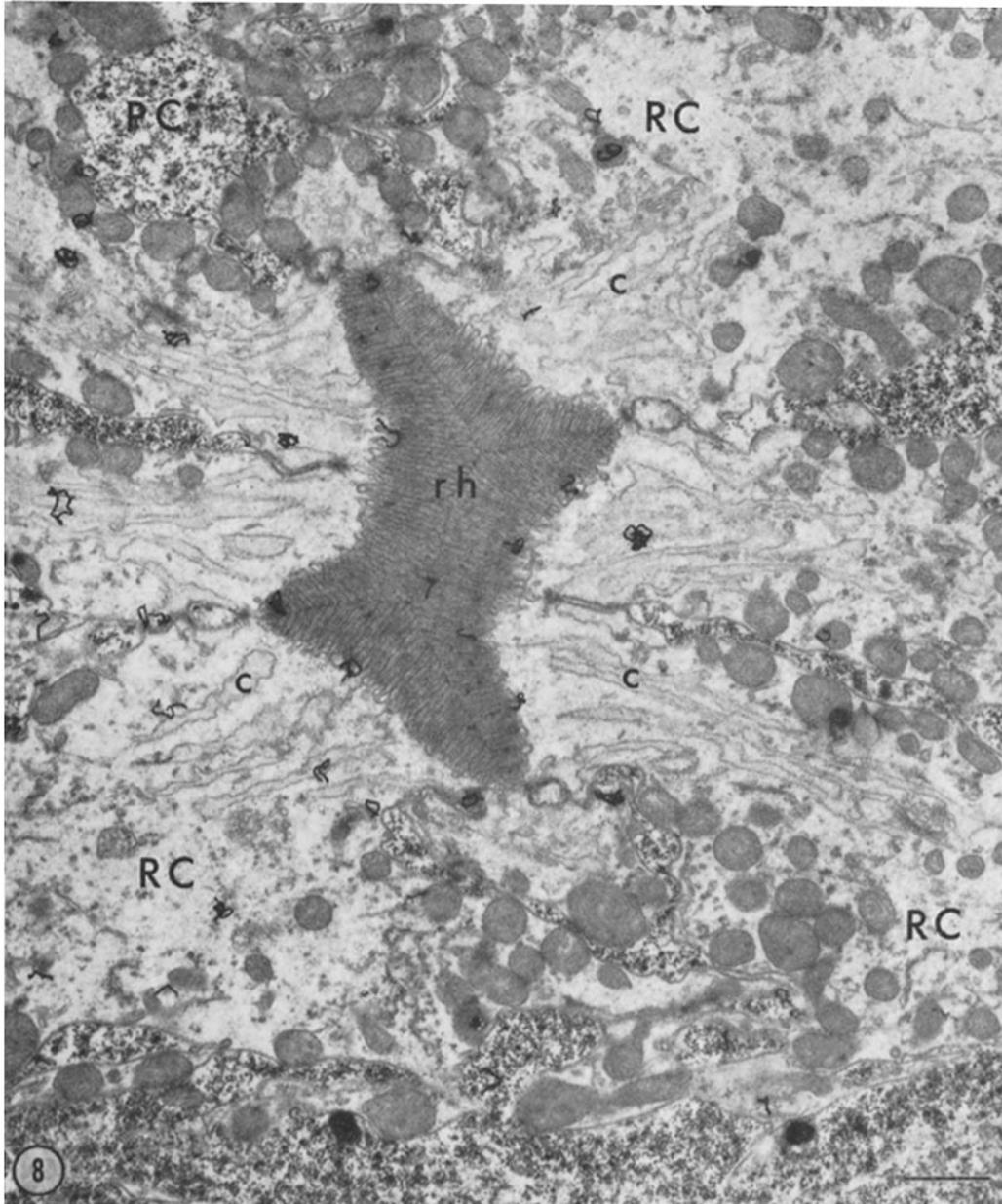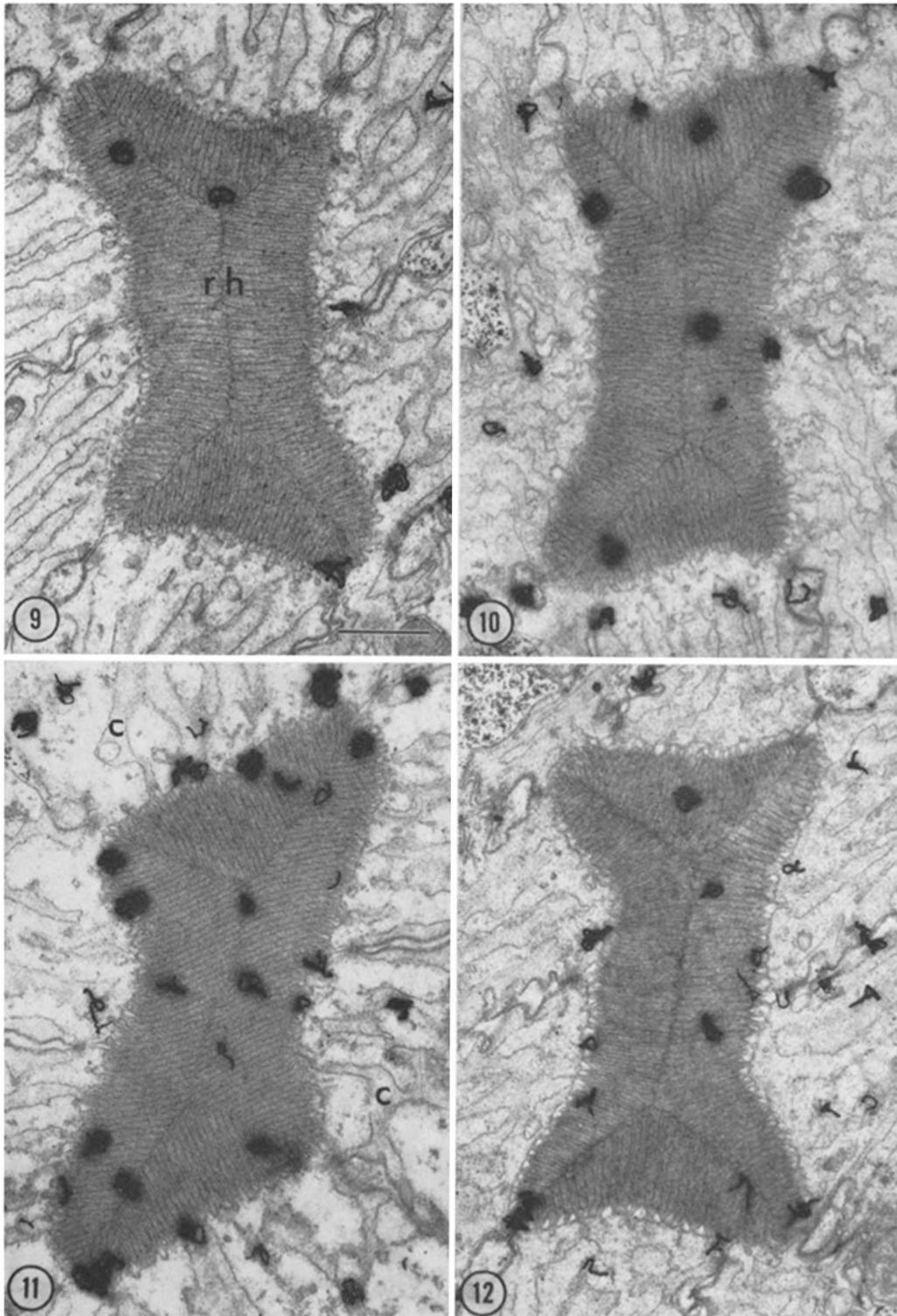

FIGURE 8   Retina fixed 96 hr after the injection of leucine. At this stage, labeling is reduced in the entire ommatidium. The reduction in labeling is more marked in the cytoplasm of the visual cells (RC) than in the rhabdomeric microvilli (rh) and the perirhabdomial region. Pigment cells (PC) are still weakly labeled. × 12,000.

FIGURES 9–12   Retinas fixed 1 hr (Fig. 9), 10 hr (Fig. 10), 48 hr (Fig. 11), and 96 hr (Fig. 12) after the injection of leucine. This set of figures shows the progressive accumulation of radioactivity within the rhabdome (rh) during the first 2 days of the experiment. Radioactivity decreases in the later stage (Fig. 12). × 14,000.

that the soluble radioactivity in the hemolymph (probably representing tritiated leucine) decreases very slowly after the injection. Tracer molecules may therefore remain available for uptake by the cells during a prolonged period of time. 96 hr after the injection of tritiated leucine (Fig. 8), radio-

activity is reduced in all three components of the visual cells, to a greater extent in the ribosome-containing cytoplasm than in the rhabdomeric microvilli or in the perirhabdomial region. In both Fig. 13 (lower graph) and in Table I, it can be seen that the radioactivity in the cytoplasm decreases

TABLE I

Distribution of Radioautographic Grains over Cell Components

| Time after injection | % of radioautographic grains ± SEM | | | | | |
	1 hr	2 hr	10 hr	24 hr	48 hr	96 hr
Peripheral cytoplasm	50.3±4.7	48.5±5.2	53.3±4.7	41.9±1.5	37.0±1.7	35.0±2.4
Perirhabdomial region	22.6±1.1	17.3±2.1	19.8±2.4	18.6±1.4	19.9±1.1	21.3±4.5
Rhabdomeric micro-villi	2.5±0.4	9.7±1.3	9.6±1.2	12.8±1.0	21.1±1.1	16.4±1.1
Golgi complex	4.6±0.6	4.0±1.0	4.3±1.1	3.1±0.5	2.9±0.2	4.2±0.5
Mitochondria	11.7±0.7	12.4±1.4	7.4±0.7	11.4±0.8	8.5±0.7	9.8±0.8
Pigment cells	8.0±0.7	8.0±1.8	5.5±0.9	12.0±0.8	9.5±0.9	13.0±1.3
No. of grains	1430	299	394	927	1650	1224
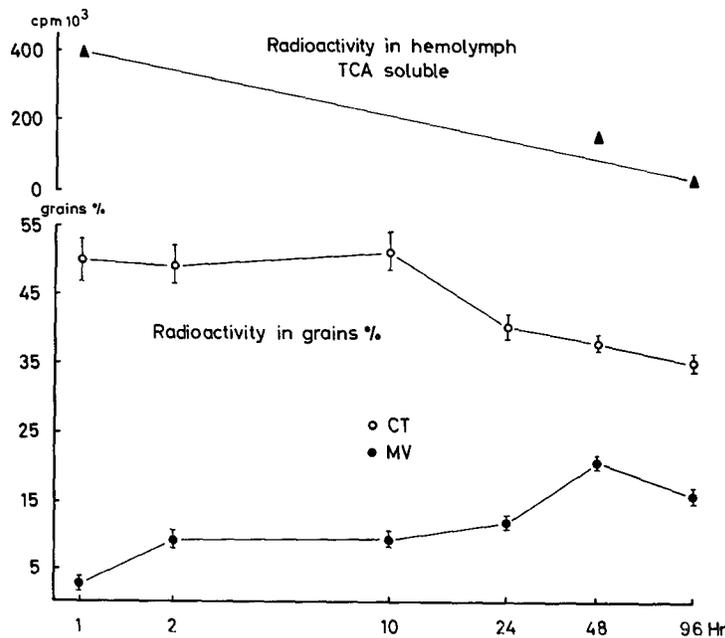
FIGURE 13 The upper graph shows the trichloroacetic acid-soluble radioactivity in the hemolymph of the drone at different intervals of time after a single injection of 80 μCi of tritiated leucine. The radioactivity was measured in a sample of 5 μl of hemolymph withdrawn from the dorsal aorta. Each point represents data derived from a different animal. The lower graph shows the evolution of radioactivity present in electron microscope radioautographs. The grain count is represented at different time intervals in two components of the visual cells: the peripheral cytoplasm (*CT*) and the rhabdomeric microvilli (*MV*). The SEM is reported for each value (vertical bars). Time is on a logarithmic scale. See Table I for grain count in other cell components.

slowly with time. The radioactivity in the rhab-
dome, on the other hand, shows the reverse: there
is very little labeling in the early stages of the ex-
periments; it then rises to a high level 48 hr after
the injection of the tracer. This finding has been
stressed in Fig. 14, where size differences of these
two cell components are taken into account (the
surface of the rhabdome is about 10% of the entire
ommatidial surface, compared with 60% for the
peripheral cytoplasm). 48 hr after the injection,
the rhabdomeric microvilli have 5 times more
radioactivity than the cytoplasm of the visual cells.
The progressive labeling of the rhabdome is shown
in Figs. 9–12. Conversely (Table I), the labeling in
the other components of the visual cells, namely
the Golgi complex, the perirhabdomial region,
mitochondria as well as the labeling of the pigment
cells surrounding the ommatidia, does not show
appreciable variations over the different periods of
time after the injection of the tracer.

DISCUSSION

The experiment described above reveals that after
a single injection of tritiated leucine in the honey-
bee drone, sizable amounts of radioactivity appear
first in the cytoplasm at the periphery of the visual
cells and later in the rhabdomeric microvilli.

Since ribosomes are present only in the periph-
eral cytoplasm of the visual cells, it seems logical
to assume that protein synthesis occurs in this cell
compartment and that the radioactivity present
in later stages in the rhabdome arises through
some migration process from the peripheral cyto-
plasm.

However, attempts to clarify further the migra-
tion process have been limited by experimental
shortcomings which shall be briefly recalled.
Firstly, the in vivo labeling of the drone retina did
not allow a true pulse experiment to be produced
(see Fig. 13, upper graph). Since labeled leucine
was continuously available to the cell for incorpo-
ration, it was not possible to visualize a single
front of newly synthesized proteins moving from
one cell compartment to another, as had been the
case in the classical pulse-labeling experiment per-
formed by Caro and Palade (3). Secondly, the
rather diffuse radioautographic reaction in the
different cell components precluded the relation
of individual silver grains to the specific organelles
which might have participated in the synthesis
and the transport of proteins. It is not known there-
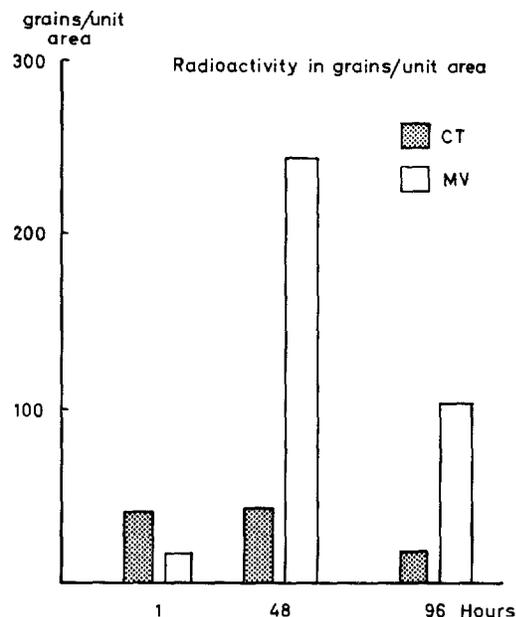fore whether protein synthesis takes place on the

FIGURE 14 The grain count of electron microscope
radioautographs expressed as a function of the respec-
tive surfaces of two main components of the visual
cells: the peripheral cytoplasm (CT) and the rhab-
domeric microvilli (MV). Surfaces were estimated by
weighing the respective areas cut out from micrographs.
Time is on a linear scale.

attached or on the free polysomes, or whether the
radioactivity in the perirhabdomial region is asso-
ciated with the cisternae or with the surrounding
cytoplasmic matrix. Finally, it seems highly likely
that we have been labeling not a single protein but
a whole set of proteins which have not necessarily
identical destinations within the cell.

Nevertheless, the unequivocal result of the ex-
periment, i.e., an early labeling of the peripheral
cytoplasm and the apparition of radioactive pro-
teins associated with the rhabdome after a period
of 24–48 hr, bears some analogy with the results
obtained by Young and Droz (16) in the retina of
the frog. In the vertebrate, the visual cell is also
compartmentalized in that the protein synthe-
sizing organelles (ribosomes) are situated in a
particular region of the cell, the inner segment,
which is distinct from the light sensitive area, the
outer segment. The outer segment is formed of
numerous discs of membrane which represent the
functional analogues of the rhabdomeric mem-
brane. After an injection of labeled amino acids
Young and Droz (16) were able to demonstrate

that radioactivity appears first in the inner segment and then migrates after a period of 4–8 hr to the outer segment. The pattern of labeling of the outer segment was found to be different in the rod and in the cone. The radioactive proteins in the rod appear as a distinct band which moves along the length of the outer segment, whereas in the cone the labeling is diffuse over the entire outer segment. The labeling of the rhabdome in the drone visual cells therefore seems to resemble that of the cone outer segment.

As already pointed out above, we do not know which proteins have been labeled by the tritiated leucine; in the frog retina, labeled proteins in the outer segment have been shown to be mainly photopigment (7). The only possible indication so far that the labeled proteins in the drone might also be, at least in part, photopigment, is that these proteins become associated with the rhabdome. If this proves true, one might ask how photopigment could become associated with the rhabdomeric membrane, since current evidence indicates that the photopigment molecules are probably embedded in the membrane bilayer (5, 15). If photopigment (protein) synthesis in our system takes place on the free polysomes of the peripheral cytoplasm, the newly synthesized molecules might diffuse through the cell sol and assemble in the rhabdomeric membrane from the inside of the microvilli. On the other hand, if synthesis occurs on attached polysomes, segregation of protein molecules should occur in the cisternal space. These proteins might be discharged in the rhabdome cavity (in between the microvilli) and perhaps assemble in the membrane from the extracellular space.

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