PEROXIDASE UPTAKE BY PHOTORECEPTOR TERMINALS OF THE SKATE RETINA

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ABSTRACT

The photoreceptors of dark-adapted skate retinas bathed in a Ringer solution containing horseradish peroxidase (HRP) incorporate the tracer into membrane-bound compartments within the synaptic terminal of the cell; after 1 or 2 h of incubation, approx. 10–38% of the synaptic vesicles were labeled. The receptors appeared to be functioning normally throughout the incubation period, since electrical potentials of normal amplitude could be elicited in response to dim photic stimuli. However, it was possible to block the uptake of peroxidase by a regimen of light adaptation that effectively suppressed light-induced activity in the electroretinogram. If, during incubation with peroxidase, retinas were exposed at 10-min intervals to an intense 1-ms flash from a xenon discharge tube, the receptor terminals were almost completely devoid of peroxidase; fewer than 2% of the vesicles were labeled. The suppression of HRP uptake could also be achieved in dark-adapted retinas by adding magnesium to the bathing solution, suggesting that calcium is necessary for transmitter release from vesicles in the receptor terminals.

These findings are consistent with the view that vertebrate photoreceptors discharge a neurotransmitter in darkness, and that light decreases the release of this substance. It seems likely that the incorporation of peroxidase into vesicles of physiologically active receptor terminals reflects a mechanism for the retrieval of vesicle membrane after exocytosis.

The absorption of light by a vertebrate photoreceptor causes the negative-resting potential of the cell to increase (30). This hyperpolarization is thought to be conducted passively to the presynaptic membrane of the receptor terminal (15), the region across which the receptor communicates with second-order neurons. While there is much indirect evidence to support the view that neurotransmission is mediated chemically (4, 10), neither the transmitter nor the mechanism by which it is released has been identified. Nevertheless, studies employing a variety of experimental approaches have yielded results which are consistent with the hypothesis that *liberation of the transmitter substance is maximal in the dark and is suppressed by light* (32).

Electrophysiological evidence favoring this notion stems mainly from analyses of the response properties of horizontal cells, a class of interneurons situated directly beneath the receptor terminals with which they make synaptic contacts (10, 33). For example, it has been shown that increas-

ing the concentration of extracellular magnesium ions causes the horizontal cell to hyperpolarize, the same response elicited by photic stimulation (10). Because it acts competitively with calcium, Mg^{++} tends to decrease the amount of transmitter substance released at chemical synapses, e.g., the cholinergic neuromuscular junction (3). Assuming that Mg^{++} produces the same effect at the receptor terminal, the hyperpolarizing response both to Mg^{++} and to light indicates that the latter exerts its effect on the horizontal cell by turning down the dark discharge of a depolarizing transmitter.

Recent histochemical studies have also provided important information as to the nature of the synaptic machinery at the photoreceptor terminal. Experiments by Schacher et al. (27) and by ourselves (26) on the uptake of horseradish peroxidase (HRP) have shown that, applied extracellularly, this protein tracer is incorporated more readily into the vesicles of dark-adapted endings than those exposed to light, i.e. the terminals exhibit greater activity in the dark than when photically stimulated.

The present paper gives a detailed account of studies in which the tracer technique was utilized to compare the effects of illumination and of excessive amounts of magnesium on the release mechanism. In addition, we have attempted to determine whether the uptake of HRP can be influenced by the change in membrane potential induced by sodium aspartate.

MATERIALS AND METHODS

Adult skate (*Raja erinacea* and *Raja oscellata*) were maintained at 14°C in opaque, aerated seawater tanks for at least 48 h before the start of an experiment. Eyes were enucleated in darkness with the aid of an infra-red light source and image converter, and pieces of eyecup (~1 cm^2) were excised from the posterior half of the globe. After removal of most of the vitreous humor with Weck-Cel sponges (E. Weck and Co., N.Y.), the tissue was mounted in a Lucite chamber and enclosed within a light-tight Faraday cage. The chamber contained a chlorided silver disc electrode which formed a platform for the scleral surface of the eyecup; a second electrode, consisting of an Ag-AgCl wire in an agar-Ringer filled pipette, made gentle contact with the retina for recording the electroretinogram (ERG).

HRP (Type II, Sigma Chemical Co., St. Louis, Mo.) was used as a tracer in a concentration of 5 mg/ml of oxygenated elasmobranch-Ringer solution; the latter contained 280 mM NaCl, 5.0 mM KCl, 4.0 mM $CaCl_2$, 2.5 mM $MgCl_2$, 2.0 mM glucose, 330 mM urea, and was buffered with $NaHCO_3$ to a pH of 7.2. In addition, experiments were conducted with perfusates in which 50 mM sodium aspartate was substituted for equimolar amounts of sodium chloride, and with calcium-deficient (1.0 mM) media in which the HRP-Ringer solution contained 20 mM $MgCl_2$. The osmolarities of these solutions were adjusted to that of the standard Ringer's (860 mosmol) by an appropriate change in the concentration of urea. Solutions were delivered to the retinal surface at a rate of 0.5 ml/min through a glass capillary tube that dipped into a well of fluid filling the eyecup; overflow (and consequent shunting of electrical currents) was prevented by suction through a second capillary tube connected to a vacuum line. Perfusion with the HRP solutions lasted for 1 or 2 h, during which time the preparations were either kept in darkness or exposed to light by one of several different procedures. In fully dark-adapted preparations, ERG recordings indicated that receptoral responses of normal amplitude (35–55 μV) could be elicited throughout the incubation period.

The light-adapting conditions were selected with the objective of suppressing fully the light-evoked electrical responses of skate rods during the time they were immersed in HRP. The slow, graded potentials generated by these cells reach a maximum amplitude, i.e., saturation, when the illuminance of a background field exceeds 2×10^{-4} mW/cm^2 (9). In the present experiments, an adapting field 100 times more intense was used either for continuous light adaptation or to stimulate the retina intermittently at a rate of 1 flash per 4 s: flash duration = 0.2 s. Alternatively, several preparations were exposed at 10-min intervals to a 1-ms flash from a xenon discharge tube adjusted in intensity so as to bleach about 20% of the available rhodopsin with each flash (7). None of these maneuvers completely eliminated the receptoral response to a bright test stimulus for the entire 2-h test period, but the xenon flash proved most efficacious in this regard (see Results).

After treatment with peroxidase, the retinas were dissected free of the choroidal and scleral coats and immersed for 7 h in cold (4°C) fixative consisting of 2% formaldehyde (prepared from paraformaldehyde) and 3% glutaraldehyde in 100 mM cacodylate buffer at pH 7.2, with 17.25% sucrose and 4.5 mM $CaCl_2$. The retinas were washed overnight in buffer, suspended in 7% agar, and cut at 40 μm with a Smith-Farquhar tissue sectioner. To demonstrate sites of peroxidase activity, the sections were treated according to a modified version (28) of the procedure introduced by Graham and Karnovsky (11). The tissue was first immersed for 30 min in 0.05% diaminobenzidine (DAB) in 0.05 M tris-HCl buffer (pH 7.6) at 20°C, and then incubated for 30 min in the complete reagent containing DAB and 0.01% H_2O_2. Postfixation was carried out in 1.3% OsO_4 in 67 mM *S*-collidine (pH 7.3) containing 17.25% sucrose. The retinas were stained *en bloc* with uranyl acetate, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (22). Thin sections were lightly stained with lead citrate and examined in a Zeiss EM 9A electron microscope.

RESULTS

The electron micrograph shown in Fig. 1, from a preparation not exposed to HRP, serves to illustrate the structural features of a skate photoreceptor terminal and its relation to neighboring elements. Although associated exclusively with rhodopsin-bearing, rodlike outer segments (7), the terminal profile does not conform to that of a typical rod spherule. A short, stubby axon joins the nuclear and synaptic regions of the cell, and an unusually large number of processes (15–25) enter the single invagination at the base of the terminal. In the sections examined, three, and often four, densely-stained lamellated bars or ribbons were frequently seen near the margin of the presynaptic membrane; it appears from the study of serial sections (6) that each ribbon is associated with postsynaptic processes that derive from horizontal and bipolar cells. Although the apposed membranes of adjacent terminals may be joined through so-called "gap" junctions (our unpublished observations; cf. also reference 35), no areas of contiguity have been observed between the photoreceptor and second-order neurons; i.e., the elements are separated by a synaptic cleft ~200 Å wide.

The cytoplasm of the receptor terminal is filled largely with electron-lucent vesicles, approx. 400–500 Å in diameter. Some of the vesicles appear to be complex or "coated" (13, 20), and there are throughout the terminal elements of endoplasmic reticulum. In addition, there is a well-defined

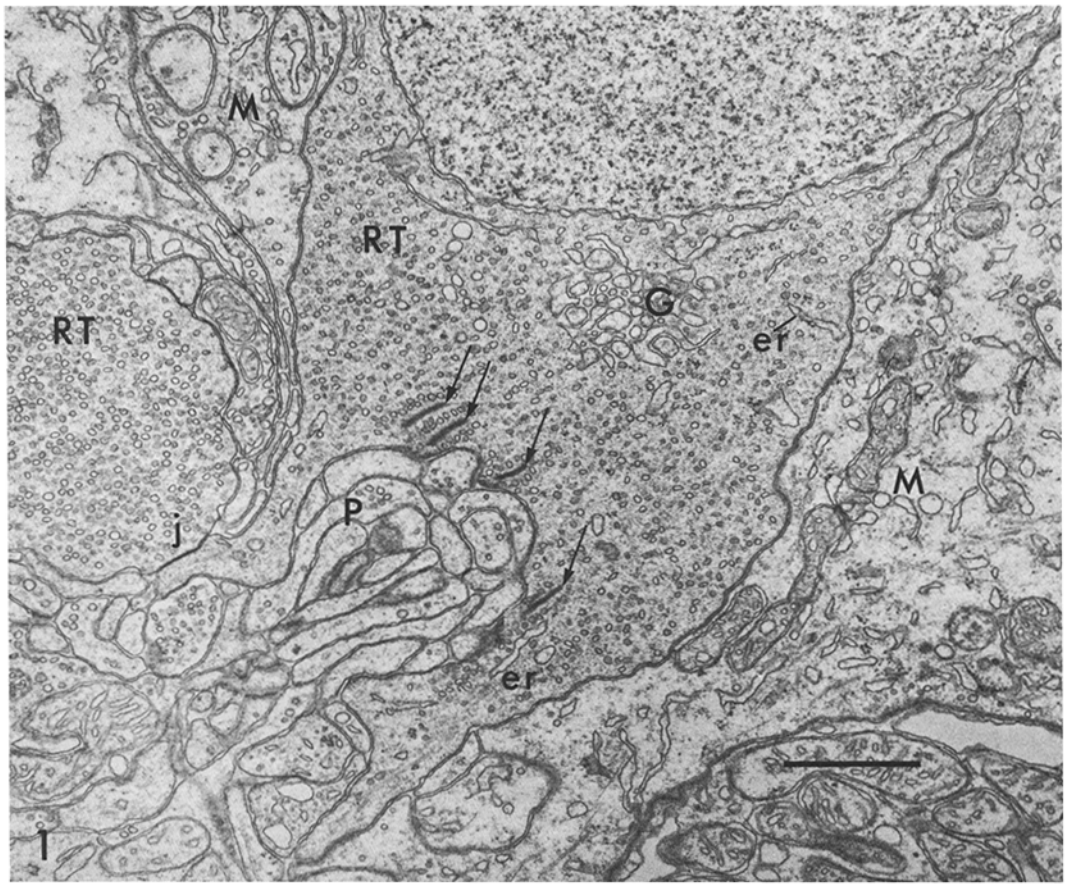

FIGURE 1 Electron micrograph of the outer synaptic region of the skate retina. The recepor terminal *(RT)* contains a large number of synaptic vesicles, several synaptic ribbons (arrows), and elements of endoplasmic reticulum *(er)*. A Golgi complex *(G)* is located near the nucleus of the cell. The processes *(P)* of second-order neurons are located within the invagination at the base of the terminal, and an interreceptoral junction *(j)* is seen between neighboring cells. Müller cell processes *(M)* are closely apposed to the receptor terminals. Sections stained with uranyl acetate and lead citrate. Scale marker, 1 μm. × 18,000.

Golgi complex located in close proximity to the nucleus, as well as a number of rounded "vacuoles" and irregularly shaped "cisternae" (cf. reference 17) whose profiles are appreciably larger than those of the vesicles. In the presynaptic region of the terminal, agranular vesicles aggregate to form a shell surrounding each ribbon. The strategic location of the ribbon and the halo of vesicles which surround it have prompted the suggestion that the ribbon serves to guide synaptic vesicles to the specific sites of the presynaptic membrane at which neurotransmitter is released (12).

Dark-Adapted Retinas

Figs. 2 and 3 show the outer synaptic regions of retinas which had been maintained in darkness for either 1 or 2 h during incubation with HRP. In each of the sections, reagent has permeated the extracellular space, and its reaction product outlines the cells and processes that occupy the external plexiform layer. Particularly noteworthy is the fact that HRP has been incorporated extensively into vesicles and other membrane-bound compartments (vacuoles and cisternae) throughout the cytoplasm of the photoreceptor terminals (Fig. 2). Reaction product was disposed in a variety of ways inside these compartments (Fig. 3), but most often it gave the appearance of inhomogeneous, irregularly shaped clumps or tufts lining the periphery of the vesicles. On the basis of examination of a large number of sections, we estimate that 10–38% (mean = 20.2%) of the vesicles contained reaction product (Table I). Some of the variability is probably attributable to the different incubation times used in these experiments, but we have not attempted to study systematically the temporal course of the response.

Reaction product was seen occasionally in the processes and cell bodies of second-order neurons and within the cytoplasm of the glial (Müller) cells. However, in addition to being relatively sparse, the uptake of HRP into these elements was not influenced by photic stimulation (see below).

Light-Adapted Preparations

The results obtained with the various light-adapting conditions showed that the uptake of HRP was markedly reduced by photic exposures. Of the various light-adapting protocols employed, that which involved intense 1-ms flashes at 10-min intervals was most effective. As shown in Fig. 4, the cytoplasm of the rod terminals is almost completely devoid of peroxidase, although reaction product outlines the perimeter of the terminal. The flash intensity, attenuated with neutral density filters, was selected on the basis of preliminary experiments to induce a prolonged saturation of the receptor potential, i.e., to suppress all light-evoked electrical activity for approx. 10 min after the flash (8). Thus, the visual cells were effectively "light-adapted" for 2 h, although the retina was in darkness for only 12 ms less than 2 h. In these circumstances, fewer than 2% of the synaptic vesicles contained reaction product (Table I).

With continuous illumination or flickering stimuli, on the other hand, skate rods "adapt" to the prevailing luminance level. Thus, the early saturating effect of a light-adapting field is soon dissipated, and the electroretinogram reveals varying degrees of photosensitivity (9, 14). In retinas which eventually become electrically responsive to test stimuli during light adaptation, some reaction product was invariably seen within the vesicles, vacuoles, and cisternae of the photoreceptor terminals, but the fraction of vesicles labeled was always significantly less than that found in dark-adapted preparations.

The Effect of Magnesium on Dark-Adapted Retinas

The photoreceptor terminals of retinas maintained in darkness for 2 h while being perfused with an HRP-Ringer solution containing an excess of magnesium ions showed a marked decrease in intracellular reaction product, despite the fact that the photosensitivity of the receptor potential was normal throughout the incubation period. As shown in Fig. 5 (see also Table I), the amount of peroxidase incorporated into vesicular compartments in these dark-adapted preparations was comparable to that found with optimal light-adapting conditions (Fig. 4).

Attempts to determine whether the ability of magnesium to block HRP uptake could be counteracted by an increase in the concentration of extracellular calcium were unsuccessful. A significant increase in calcium ($[Ca]_o \geq 20$ mM) reduced the amplitude of the receptor potential to below the noise level of the recording apparatus, a consequence most probably of its hyperpolarizing effect on the cell's membrane potential (see Discussion).

Sodium Aspartate and Peroxidase Uptake

The previous results provide further evidence that the release of transmitter from the visual cell is maximal in the dark; the adequate stimulus for

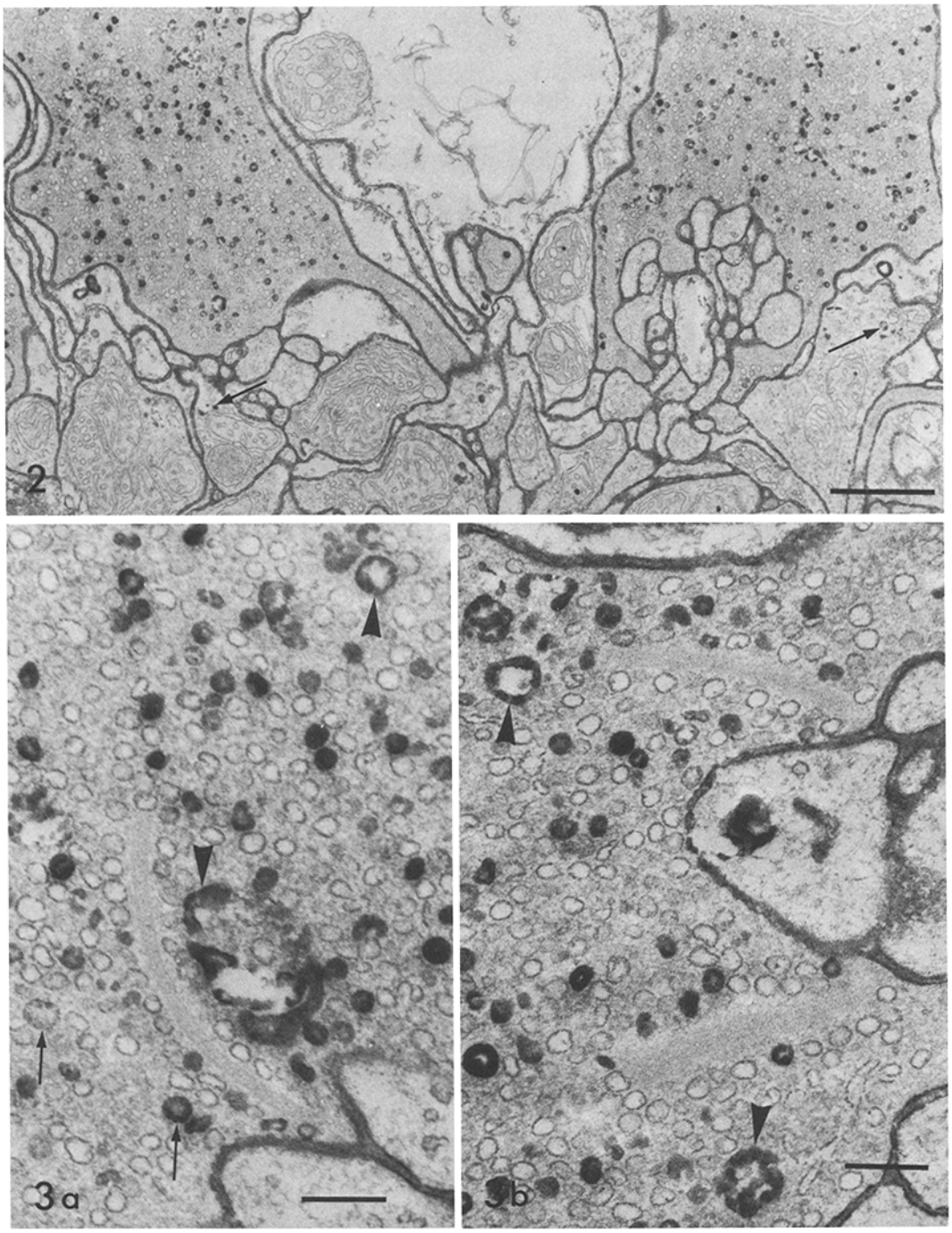

FIGURE 2 Electron micrograph of the outer synaptic region of a retina incubated in Ringer's with HRP for 1 h in darkness. Many synaptic vesicles, vacuoles, and cisternae containing peroxidase reaction product are located throughout the terminals, but the tracer is seen only rarely in nonreceptoral elements (arrows). In these terminals, approx. 35% of the vesicles contain HRP. Reaction product is also present in the extracellular spaces outlining the cells and their processes. Scale marker, 1 μm. × 15,300.

FIGURE 3 Electron micrographs of photoreceptor terminals from a dark-adapted preparation incubated for 2 h in HRP-Ringer's. Reaction product appears as inhomogeneous, irregularly shaped clumps of electron-dense material lining or filling the vesicles, as well as the larger cisternae and vacuoles (arrowheads). A few complex or "coated" vesicles are also seen (arrows). Scale marker, 0.2 μm. × 62,000.

TABLE I

The Effect of Photic and Ionic Conditions on Uptake of HRP

Experimental condition	Retinas	Terminals counted*	HRP-labeled vesicles‡
			%
Dark adapted	12	60 ($\bar{n}$ = 516)	20.2 ± 1.8
Flash (1 ms)/10 min	8	40 ($\bar{n}$ = 498)	1.4 ± 0.5
$[Mg^{++}]_o$ increased	7	35 ($\bar{n}$ = 480)	2.9 ± 1.1
Na aspartate	9	45 ($\bar{n}$ = 530)	26.7 ± 2.7

$\bar{n}$ gives the average number of vesicles per section.
* One micrograph of each receptor terminal was selected at random.
‡ Mean ± standard error of the mean.

the receptor (namely, light) can only decrease the rate at which transmitter is liberated. Nevertheless, in an attempt to enhance transmitter release and to determine whether the uptake of peroxidase was modulated by the membrane potential of the photoreceptor, we added sodium aspartate to the HRP-Ringer solution bathing the retina. Kleinschmidt (21) has shown with intracellular recordings from *Gekko* rods that aspartate depolarizes the cell membrane and increases the maximum voltage which the receptor can generate in response to light.

Fig. 6 shows receptor terminals from dark-adapted retinas that had been perfused for 2 h with the aspartate-Ringer solution. It appeared that the intracellular accumulation of reaction product was somewhat greater than that seen in dark-adapted retinas bathed in solutions which did not contain aspartate (Fig. 2). In addition, aspartate seems to have induced the formation of a greater number of vacuoles and cisternae, most of which exhibit peroxidase activity. In a large number of sections, reaction product was concentrated mainly in the region of the Golgi complex where there appeared to be a confluence of vesicles and cisternae. Although it seemed that a greater amount of vesicular membrane was labeled by peroxidase (Table I), we did not find an increased accumulation of reaction product within vesicles surrounding the presynaptic ribbon or along the presynaptic membrane.

DISCUSSION

The findings reported here, and those of Schacher et al. (27), show clearly that dark-adapted vertebrate photoreceptors take up peroxidase from the extracellular space and incorporate the tracer into membrane-bounded compartments within their synaptic terminals (Figs. 2 and 3). Light adaptation sufficient to make the receptor refractory to further stimulation suppresses almost completely the uptake of peroxidase (Fig. 4). Since reaction product is found almost exclusively within vesicular elements, and not elsewhere in the cytoplasmic matrix, it appears likely that the uptake results from an endocytotic process that follows the discharge of neurotransmitter in the dark.

It has been suggested that an exocytosis-endocytosis coupling system provides the means by which secretory cells conserve their membrane constituents (2, 5, 19, 23). Using the peroxidase tracer technique to follow the sequence of events involved in this process, Heuser and Reese (17) have proposed a mechanism by which rapid recycling occurs at the motor nerve terminal of the frog. According to their scheme, vesicles release their transmitter by exocytosis at particular sites on the presynaptic membrane. The vesicular membranes then move within the fluid phase of the plasma membrane, and some distance away begin to reform as "coated" vesicles. These, in turn, coalesce to produce large cisternae, and membranous buds pinch off from the cisternae to form new vesicles; evidence that the newly formed vesicles can store and release transmitter was presented by Ceccarelli et al. (2). Although we have not attempted to determine the temporal course taken in the translocation of peroxidase at the photoreceptor terminal, it is noteworthy that reaction product was confined to elements implicated by Heuser and Reese in the recycling process, namely, synaptic vesicles, coated vesicles and cisternae (Fig. 3). Also of interest in this connection is the large accumulation of reaction product found within cisternae after depolarization of the receptor with sodium aspartate (Fig. 6). However, even in those terminals in which a great deal of HRP was taken up, we did not observe an increase in the number of peroxidase-filled vesicles at presumptive release sites along the presynaptic membrane. It is possible that the membrane-retrieval mechanism is not the main route used by intact photoreceptors to provide neurotransmitter-charged vesicles. On the other hand, the presence of labeled vacuoles and cisternae in the dark-adapted state implies, according to the Heuser-Reese scheme, that vesicle membrane is already being recovered faster than it can be turned into synaptic vesicles. Thus, the effect of inducing even

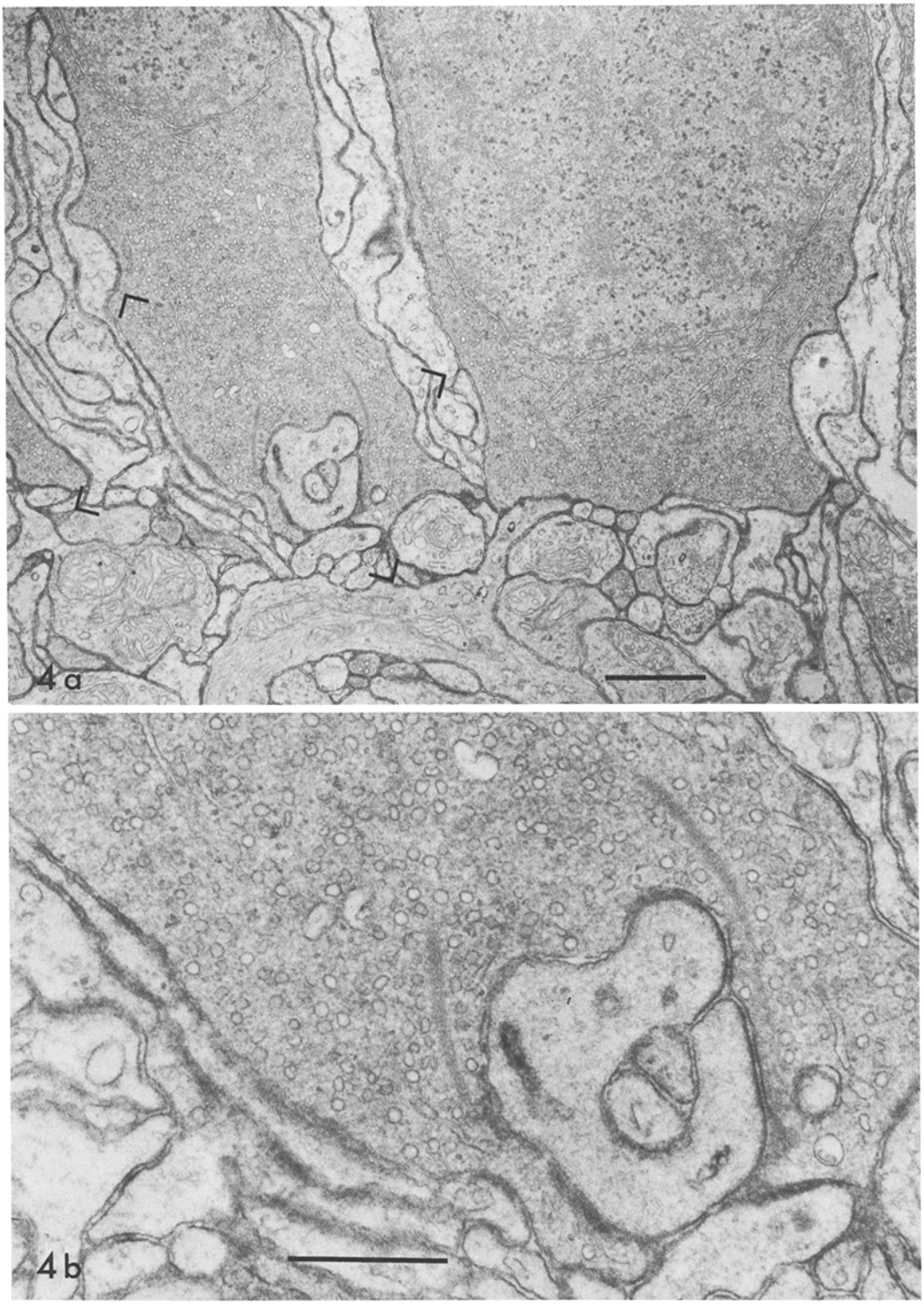

FIGURE 4 *(a)* Electron micrograph of outer synaptic region of a retina incubated in HRP and exposed to 1-ms xenon flashes at the rate of 1 flash every 10 min for 2 h. The synaptic vesicles are devoid of peroxidase, although reaction product does appear in the extracellular space. Scale marker, 1 μm. × 15,300. *(b)* Higher magnification of the region outlined in *(a)*. Scale marker, 0.5 μm. × 48,500.

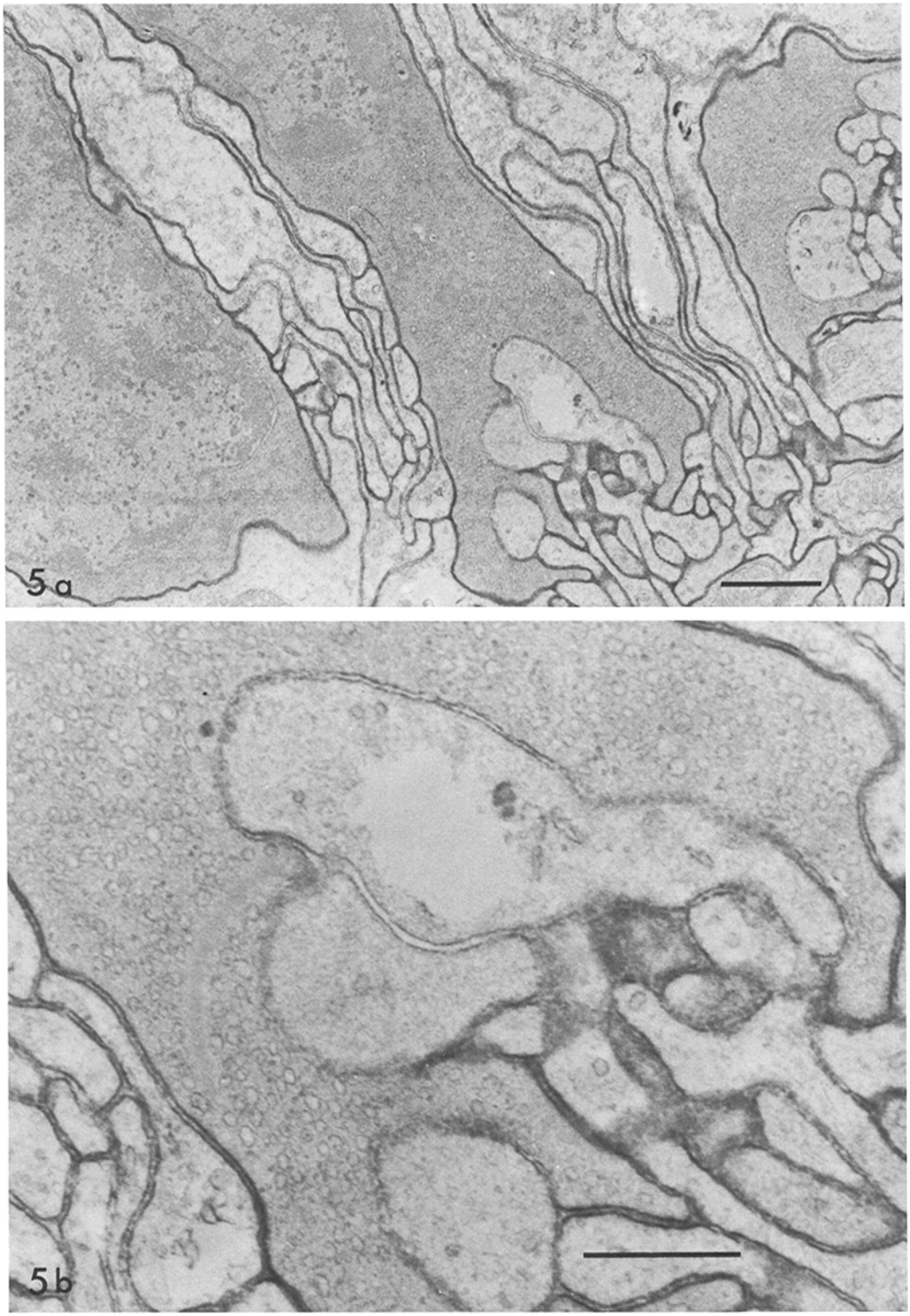

FIGURE 5 Electron micrographs of the outer synaptic region of a retina incubated for 1 h in darkness in low calcium Ringer's containing 20 mM $MgCl_2$ and HRP. The appearance of the receptor terminals is similar to that of the light-adapted preparation (Fig. 4), in that reaction product is rarely seen within the synaptic vesicles, but is present in the extracellular space. *(a)* Scale marker, 1 μm. × 15,300. *(b)* Scale marker, 0.5 μm. × 48,500.

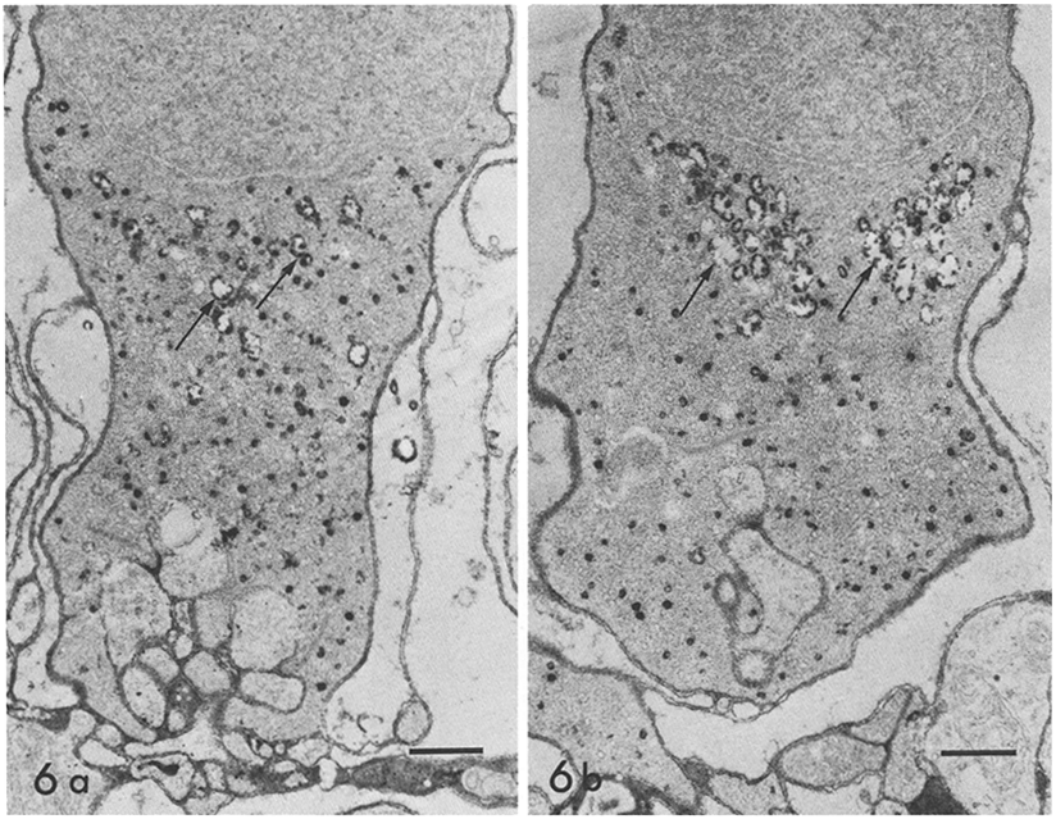

FIGURE 6 Electron micrographs of photoreceptor terminals of a retina that was incubated for 2 h in darkness in Ringer's containing 50 mM sodium aspartate and HRP. Counts taken from enlarged micrographs indicate that in *(a)* about 27% of the synaptic vesicles and almost 83% of the vacuoles and cisternae contain reaction product; in *(b)* the corresponding values are 23% and 86%, respectively. Many of the tracer-labeled vacuoles and cisternae (arrows) are present in the region of the Golgi complex. Scale marker, 0.5 μm. × 19,500.

more vesicle discharge and recovery may tend to increase the number of cisternae within the terminal.

The membrane potential of the dark-adapted photoreceptor is also of interest with regard to the mechanism by which the cell controls the discharge of its neurotransmitter. There is compelling evidence to indicate that in darkness a steady current flows inward across the plasma membrane of the receptor outer segment, and outward through the membrane of more proximal regions of the cell (25). It is generally agreed that the principal ionic species responsible for this dark current is sodium, and that, by a mechanism yet to be elucidated, quantal absorption in the outer segment reduces the influx of Na^+ and causes the cell to hyperpolarize (1, 29). Thus, the resting potential of the dark-adapted cell (−30 mV) is considerably less than the voltage level attained during photic stimulation (31, 34). The foregoing remarks point to the fact that, in so far as transmitter release is concerned, the photoreceptor terminal behaves in an orthodox fashion. As with other neurosecretory cells, depolarization enhances and hyperpolarization suppresses the liberation of transmitter substances.

A question we have not been able to resolve fully concerns whether the release of transmitter depends upon the entry of calcium into the photoreceptor terminal. The fact that magnesium can effectively block the uptake of peroxidase (Fig. 5), as well as cause horizontal cells to respond with the same polarity as that elicited by photic stimulation, indicates that an excess of Mg^{++} upsets the normal dark release of transmitter. But there is no assurance that these reactions

result from interference with a calcium-dependent process.

A convenient way of demonstrating that competitive inhibition is responsible is to reconstitute the release mechanism in the presence of Mg^{++} through the addition of Ca^{++} (3). This maneuver could not be adopted in the present situation (see Results). We noted earlier that a continuous dark current is generated by an influx of Na^{+} into the outer segments of the visual receptor, and that light decreases this sodium conductance. The results of recent studies suggest that the dark current is controlled by the activity of calcium ions, i.e., an increase in Ca^{++} concentration mimics the effect of light in that it hyperpolarizes the photoreceptor and decreases the receptor's response to photic stimuli (1, 16, 36). Irrespective of whether calcium is an intermediary between photon absorption and the reduction in membrane permeability to sodium, it seems likely that the inhibitory effect exerted by excess Ca^{++} at the distal end of the photoreceptor would offset whatever increase it might produce in vesicle turnover (and peroxidase uptake) at the synaptic terminal. An alternative approach to this problem which we are currently exploring involves the use of EM and X-ray dispersive analysis to identify calcium-binding sites within the receptor terminal (18, 24).

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REFERENCES

1. Brown, J. E., and L. H. Pinto. 1974. Ionic mechanism for the photoreceptor potential of the retina of *Bufo marinus*. *J. Physiol. (Lond.).* **236:**575-591.
2. Ceccarelli, B., W. P. Hurlbut, and A. Mauro. 1973. Turnover of transmitter and synaptic vesicles at the frog neuromuscular junction. *J. Cell Biol.* **57:**499-524.
3. Del Castillo, J., and L. Engbaek. 1954. The nature of the neuromuscular block produced by magnesium. *J. Physiol. (Lond.).* **124:**370-384.
4. De Robertis, E., and C. M. Franchi. 1956. Electron microscopic observations on synaptic vesicles in synapses of the retinal rods and cones. *J. Biophys. Biochem. Cytol.* **2:**307-317.
5. Douglas, W. W., J. Nagasawa, and R. A. Schultz. 1970. Electron microscopic studies on the mechanism of secretion of posterior pituitary hormones and the significance of microvesicles ('synaptic vesicles'): evidence of secretion by exocytosis and formation of microvesicles as a by-product of this process. *Mem. Soc. Endocrinol.* **19:**353-377.
6. Dowling, J. E. 1974. Synaptic arrangements in the vertebrate retina: the photoreceptor synapse. *In* Synaptic Transmission and Neuronal Interaction. M. V. L. Bennett, editor. Raven Press, Inc., New York. 87-103.
7. Dowling, J. E., and H. Ripps. 1970. Visual adaptation in the retina of the skate. *J. Gen. Physiol.* **56:**491-520.
8. Dowling, J. E., and H. Ripps. 1971. S-potentials in the skate retina: intracellular recordings during light and dark adaptation. *J. Gen. Physiol.* **58:**163-189.
9. Dowling, J. E., and H. Ripps. 1972. Adaptation in skate photoreceptors. *J. Gen. Physiol.* **60:**698-719.
10. Dowling, J. E., and H. Ripps. 1973. Effect of magnesium on horizontal cell activity in the skate retina. *Nature (Lond.).* **242:**101-103.
11. Graham, R. C., and M. J. Karnovsky. 1966. The early stage of absorption of injected horseradish peroxidase in the proximal tubule of the mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14:**291-302.
12. Gray, E. G., and H. L. Pease. 1971. On understanding the organization of the retinal receptor synapses. *Brain Res.* **35:**1-15.
13. Gray, E. G., and R. W. Willis. 1970. On synaptic vesicles, complex vesicles and dense projections. *Brain Res.* **24:**149-168.
14. Green, D. G., and I. M. Siegel. 1975. Double branched flicker fusion curves from the all-rod skate retina. *Science (Wash. D. C.).* **188:**1120-1122.
15. Hagins, W. A. 1972. The visual process: excitatory mechanisms in the primary receptor cells. *Annu. Rev. Biophys. Bioeng.* **1:**131-158.
16. Hagins, W. A., and S. Yoshikami. 1974. A role for Ca^{2+} in excitation of retinal rods and cones. *Exp. Eye Res.* **18:**299-305.
17. Heuser, J. E., and T. S. Reese. 1973. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. Cell Biol.* **57:**315-344.
18. Hillman, D. E., and R. Llinas. 1974. Calcium-containing electron-dense structures in the axons of the squid giant synapse. *J. Cell Biol.* **61:**146-155.

19. HOLTZMAN, E., A. R. FREEMAN, and L. A. KASHNER. 1971. Stimulation-dependent alterations in peroxidase uptake at lobster neuromuscular junctions. *Science (Wash. D. C.).* **173:**733–736.
20. KANASEKI, T., and K. KADOTA. 1969. The vesicle in a basket. *J. Cell Biol.* **42:**202–220.
21. KLEINSCHMIDT, J. 1973. Adaptation properties of intracellularly recorded Gekko photoreceptor potentials. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, New York. 219–224.
22. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* **9:**409–414.
23. ORCI, L., F. MALAISSE-LAGAE, M. RAVAZOLLA, and M. AMHERDT. 1973. Exocytosis-endocytosis coupling in the pancreatic beta cell. *Science (Wash. D. C.).* **181:**561–562.
24. OSCHMAN, J., and B. WALL. 1972. Calcium binding to intestinal membranes. *J. Cell Biol.* **55:**58–73.
25. PENN, R. D., and W. A. HAGINS. 1969. Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature (Lond.).* **223:**201–205.
26. RIPPS, H., M. SHAKIB, and E. D. MACDONALD. 1974. Turnover of synaptic vesicles in photoreceptor terminals of the skate. *Biol. Bull. (Woods Hole).* **147:**495.
27. SCHACHER, S. M., E. HOLTZMAN, and D. C. HOOD. 1974. Uptake of horseradish peroxidase by frog photoreceptor synapses in the dark and the light. *Nature (Lond.).* **249:**261–263.
28. SHAKIB, M., G. N. WISE, and P. RUTKOWSKI. 1972. Fluorescein angiography and the retinal pigment epithelium. *Am. J. Ophthalmol.* **74:**206–218.
29. SILLMAN, A. J., H. ITO, and T. TOMITA. 1969. Studies on the mass receptor potential of the isolated frog retina. II. On the basis of the ionic mechanism. *Vision Res.* **9:**1443–1451.
30. TOMITA, T. 1965. Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp. Quant. Biol.* **30:**559–566.
31. TOYODA, J., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vision Res.* **9:**453–463.
32. TRIFONOV, YU. A. 1968. Study of synaptic transmission between the photoreceptor and the horizontal cell using electrical stimulation of the retina. *Biofizika* **13:**809–817.
33. TRIFONOV, YU. A., A. L. BYZOV, and L. M. CHAILAHIAN. 1974. Electrical properties of subsynaptic and nonsynaptic membranes of horizontal cells in fish retina. *Vision Res.* **14:**229–241.
34. WERBLIN, F. S. 1975. Regenerative hyperpolarization in rods. *J. Physiol. (Lond.).* **244:**53–81.
35. WITKOVSKY, P., M. SHAKIB, and H. RIPPS. 1974. Interreceptoral junctions in the teleost retina. *Invest. Ophthalmol.* **13:**996–1009.
36. YOSHIKAMI, S., and W. A. HAGINS. 1971. Light, calcium, and the photocurrent of rods and cones. *Biophys. Soc. Annu. Meet. Abstr.* **11:**47.