Submicroscopic Changes in Visual Cells of the Rabbit Induced by Iodoacetate*†

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ABSTRACT

Alterations produced by iodoacetate in visual cells have been studied under the electron microscope. Lesions of the outer segments of the rods are visible as early as 3 hours after a single injection of 20 mg. iodoacetate per kg. body weight. After 6 hours the changes are more marked and consist then of disorganization, vesiculation, and lysis of the rod sacs. The inner segments of most rod cells show swelling and vacuolization of the matrix, the endoplasmic reticulum, and the Golgi complex. The mitochondria of the ellipsoid show a tendency to disintegrate. In some inner segments the changes consist primarily in an increase in density of the matrix and deposition of a granular material. The rod synapses are also affected, showing lysis of the synaptic vesicles and alterations of the synaptic membrane.

With a second injection of 20 mg. iodoacetate per kg. body weight, all these changes become more marked and lead to complete destruction of the rod cells.

The cones seem more resistant than the rods. A single injection produces no visible changes in the outer or inner segments of the cones. At cone synapses, however, there are changes consisting of fusion of synaptic vesicles and other membranous material to form large concentric membranes characteristic of myelin figures. A second dose of the drug causes complete destruction of the cone cells.

All these, and other submicroscopic changes, are discussed in relation to various hypotheses put forward to explain the mode of action of iodoacetate on visual cells. The pronounced alterations of submicroscopic intracellular membranes suggest that the locus of action of iodoacetate may be a component widely dispersed throughout the visual cells and related, in some way, to the maintenance of these lipoprotein structures.

INTRODUCTION

Since Noell (2) described electroretinographic changes following intravenous injection of iodoacetic acid, much work has been done to determine the morphologic bases of these functional alterations which may lead to permanent blindness. Definite lesions of the visual cells were first observed in the retina of the rabbit (3). According to Noell (4, 5), functional effects that are reversible

occur in most cats and rabbits given a single injection of 12 to 20 mg. per kg. body weight. A second dose produces definite degenerative changes in visual cells and picnosis follows in 8 to 12 hours. The final histologic picture shows almost total destruction of visual cells, while the integrity of most of the other retinal strata is preserved. In monkeys, cones appear to be more resistant than rods to the action of the drug (4, 5). All later studies (6 through 10) have confirmed the selective effects of iodoacetate on visual cells. Histochemical studies in cock retina have shown that changes in glycogen, lipides, and nucleic acids follow the structural alterations (11).

These methodologic approaches, including the study of metabolic alterations in the retina (12),

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fail to provide a full understanding of the degenerative mechanism in visual cells; hence the excuse for further investigations. In the present study, the earliest alterations induced by iodoacetic acid are studied with the electron microscope. A variety of submicroscopic changes occurring at different visual cell segments are described, and then discussed in connection with several hypotheses advanced to explain the mechanism of action of iodoacetate on photoreceptors.

Techniques

Iodoacetic acid was dissolved in distilled water, neutralized with sodium hydroxide, and then immediately injected intravenously in rabbits in a dose of 20 mg./kg. body weight. In one group of animals, no further injections were given, and the retinas were fixed after 3 and 6 hours. In another group, a second injection, equal to the first, was given after 6 hours, and the retinas fixed 3, 12, 18, and 24 hours later.

The rabbits were anesthetized with nembutal and an incision made beyond the sclerocorneal limbus. The vitreous was removed and fixative was dropped directly into the ocular cavity. In this way, a substantial part of the fixation was accomplished *in situ*, with nervous and vascular connections intact. After extraction of the eye, pieces of retina were put into fresh solution of fixative at 0°C. An $\text{OsO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ solution (Dalton's solution (13)) and an isosmotic osmium fixative containing polyvinylpyrrolidone and balanced ions (14) were used. Pieces of tissue were oriented so as to obtain sections perpendicular to the surface of the retina, and after embedding in butylmethacrylate, were sectioned with a Porter-Blum microtome. The sections were observed with an RCA-2E electron microscope with a 50 μ aperture in the objective. Residual astigmatism was compensated with a Canalco stigmator.

OBSERVATIONS

Under the present experimental conditions, alterations occur exclusively in the visual cells; no changes can be discerned in the other cellular elements of the retina. The first and most interesting submicroscopic lesions are localized in the outer and inner segments of the rods and at the synaptic region of both visual cells. (For a description of the normal ultrastructure of photoreceptors in the rabbit, see references 15 and 16).

Submicroscopic Changes in the Outer Segments of Rod and Cone Cells:

As early as 3 hours after a single injection of iodoacetate, changes occur in the outer segments. The typical normal multilamellar structure—with the regularly spaced rod sacs—appears dis-

organized, with regions in which the sac membranes are wavy and irregularly spaced. Furthermore, in localized regions of the outer segments, the disintegration of the membranous structure leads to the formation of spaces containing an amorphous or granular material.

Six hours after a single injection, these changes appear more pronounced. Fragmented and dilated rod sacs can be seen, and these result in the formation of numerous vesicular structures. The characteristic degenerative changes are shown in Fig. 2. The multilamellar structure is maintained only in small regions of the outer segment. Large vesicles or granules, the results of rod sac fragmentation, occupy the remaining area.

In the retinal cells of rabbits given a second injection, alterations of the outer segment are more marked and lead to disintegration of the periodic structure and destruction of the surface membrane (Fig. 6). Changes are not visible in the outer segments of the cones at the stage of primary damage in the rods, but in the advanced stages, when the outer segments of the rods are totally disorganized, the cones are damaged almost beyond recognition.

Submicroscopic Changes in the Inner Segments of Rod and Cone Cells:

Changes in the inner segment can be seen 6 hours after a single injection of iodoacetate and appear to be of two types. In some rod cells, pronounced vacuolization predominates, and the segment appears less dense, rather spongy, and swollen (Fig. 3, *VRIS*). In other rod cells the structure is more compact and the density of the segment is heightened (Fig. 3, *DRIS*).

In the first case (which is the more common), the vacuoles appear as clear spaces within the cytoplasmic matrix and frequently show a definite limiting membrane (Figs. 3 and 4). Some vacuoles seem to be related to the endoplasmic reticulum or to the Golgi complex. Although vacuolization of the cytoplasm is the major alteration, small intramitochondrial vacuoles and signs of disintegration into smaller dense bodies may also appear in the mitochondria of the ellipsoid (Fig. 3).

The second and less frequent type of alteration of the inner segment consists of an increase in the density of the cytoplasmic matrix and an accumulation of a dense particulate material. In this case mitochondria are more packed than in the previously described segments. A few vesicles can be seen within the dense cytoplasmic matrix

in Figs. 3 and 5. After the second injection, these two types of alterations become more conspicuous, leading in one case to vacuolar destruction of the hyaloplasm and disintegration of the mitochondria and surface membrane (Fig. 6), and in the other case to extreme condensation and contraction of the entire segment.

The connecting cilium (1, 5) does not show early changes; its disintegration is contemporaneous with the destruction of the outer and inner segments.

The inner segment of the cone cells appears intact in the early stages. However, as is the case with the outer segment, it is not recognizable when the lesions of the visual cells are more advanced.

Submicroscopic Changes in the Synapse of Visual Cells:

Both the spherule of the rod and the pedicle of the cone show conspicuous changes. In the rod spherule there is a definite diminution in the number of normal synaptic vesicles as early as 6 hours after one injection. This change becomes intense after the second injection, and in many spherules most of the synaptic vesicles are destroyed and then disappear (Fig. 9). At the same time, the two adjacent membranes that constitute the synaptic membrane are badly distorted.

In the cone, the changes are of a different nature. In the early period, the synaptic vesicles are maintained (Fig. 7), and the synaptic membrane remains intact. The most characteristic change, shown in Figs. 7 and 8, consists in the formation of concentric membranous structures resembling myelin. These "myelin figures" result from the intracellular fusion of different sized vesicles. The process leads to the formation of multimembranous structures that, at the outset, have a disoriented, honeycomb appearance (Fig. 7). In other cone pedicles, these membranes, which are about 30 Å thick, become more regularly spaced and are disposed in pairs separated by 50 to 60 Å spaces. At this stage the membranes assume a definite concentric configuration characteristic of myelin figures (Fig. 8).

DISCUSSION

Iodoacetic acid has been widely used as an inhibitor of some enzyme systems. The drug acts on glycolysis by inhibiting phosphoglyceraldehyde dehydrogenase. At high concentrations, it may also affect some enzymes of the Krebs cycle (17). The blocking action of iodoacetic acid on sulfhydryl

groups is also well known, but its sensitivity and specificity is not so marked as that of other agents that form mercaptide linkages (18). These and other properties of iodoacetic acid have been mentioned to suggest the mechanism of its action on the visual cells.

The blocking of SH groups of proteins cannot explain satisfactorily the selective destruction of visual cells without damaging other retinal strata. Furthermore, Sorsby *et al.* (19), using a wide variety of sulfhydryl reagents, some of which are more sensitive and specific than iodoacetic acid, found that only this drug produces retinal lesions.

Similar arguments may be used to dispose of the hypothesis that attributes the degeneration of visual cells to an interference with the rhodopsin cycle. Wald and Brown (20), using *p*-chloromercuribenzoate *in vitro*, demonstrated the importance of SH-groups in the synthesis of rhodopsin. However, iodoacetic acid has no effect *in vitro* on this synthesis. Since rhodopsin is localized only in the outer segment of the rod, it would also be difficult to explain, with this hypothesis, the total destruction of the visual cell (5).

The action of iodoacetic acid on glycolysis seems to be more important. In fact, the retina has the highest rate of glycolytic activity (5), localization being predominant at the visual cells (1, 12), and the effect of iodoacetic acid has been demonstrated both *in vitro* and *in vivo* (21, 22). These observations support the hypothesis that suppression of glycolysis could lead to functional impairment and ultimately to the death of the visual cell (4, 5, 12). This interpretation granted, it still remains to be explained why other inhibitors of glycolysis, such as flordizine and sodium fluoride, do not produce retinal lesions (9, 10).

Our results indicate that iodoacetate acts simultaneously at the different segments of the rod cells. Although 3 hours after a single dose only the outer segments appear to be affected, after 6 hours, effects are evident in the inner segment and synaptic region as well. This wide extension of the first submicroscopic changes supports the view that iodoacetate acts on enzymes (or other cell components) widely distributed in the rod cell and not localized in a single segment (rhodopsin). In this respect, the phosphoglyceraldehyde dehydrogenase appears to be present in the entire visual cell, although its concentration is relatively low in the outer segment (23). The enzymes of the Krebs cycle, on which iodoacetate may also act (17), are found exclusively at the inner segment,

where all the mitochondria of the rabbit visual cell are concentrated (15, 16).

The early lesions of the rod cells described here, produced with a single dose of iodoacetate, belong to the period in which no histologic changes can be observed with an optical microscope. The ultrastructural alterations coincide with the transient functional disturbance of the visual cells (4, 5). We have not sought to determine if the electroretinographic recovery in these cases is coincident with restoration of all or part of the visual cell population.

The lesions found after a second injection are more widespread and intense than, although similar to, the early ones, and belong to the period of irreversible functional changes that leads to destruction of all visual elements, and permanent blindness in the animal.

Another point of contention concerns the different sensitivity to iodoacetate shown by rods and cones. In the eye of the monkey, Noell described (4, 5) the greater sensitivity of the rod, but he could not find this difference in rabbits. In our observations we find that, with a single dose, the cone cells appear unaltered except at some synaptic pedicles, where damages do occur and lead to formations reminiscent of "myelin figures." This is in contrast to those rod cells that show important changes at their outer and inner segments and at the spherule. With a second dose of iodoacetate both rod and cone cells are destroyed.

Regarding the early lesions of the rod cells, it is interesting to note that there are two types of reactions—the more common one leading to a swelling and vacuolization of the inner segment, and the other to its retraction and condensation. We may speculate that this different reactivity may be the result of some special difference in chemical organization within an apparently single type of rod cell.

The formation of myelin figures by a process of fusion of synaptic vesicles (24) and of other membranous material present in the cone pedicle is of considerable interest. Multilamellar structures have been seen to form within chloroplasts in normal plant cells by a process of vesicle-fusion (25), during morphogenesis of the outer segment of retinal rods (26), and in myelinogenesis of the central nervous system (27). In experimental silicosis, concentric systems of membranes have

been observed within lung cells (28). Recently, Fawcett and Ito (29) followed the formation of myelin figures in testicular cells maintained in saline with phase and electron microscopes. In this instance, membranes resulted from material derived from the endoplasmic reticulum.

The finding of these multilamellar structures in the cone pedicle together with the destructive changes of the synaptic vesicles, the alterations of the membranes of the endoplasmic reticulum and Golgi complex, and finally, the lesions of the membranes of the rod sacs, all indicate that iodoacetate acts predominantly at the level of most intracellular membranes of the visual cells. It seems reasonable to think that the locus of action of iodoacetate may be a component present in these different lipoprotein membranes or connected with the maintenance of the intracellular membranes of visual cells.

BIBLIOGRAPHY

1. Lasansky, A., and De Robertis, E., *Anat. Rec.*, 1958, **130**, 423.
2. Noell, W. K., *J. Cell. and Comp. Physiol.*, 1951, **37**, 283.
3. Schubert, G., and Bornschein, H., *Experientia*, 1951, **7**, 461.
4. Noell, W. K., *J. Cell. and Comp. Physiol.*, 1952, **40**, 25.
5. Noell, W. K., United States Air Force School of Aviation Medicine, Project No. 21-1201-0004, Report No. 1, 1953.
6. de Berardinis, E., and Bonavolontà, G., *Boll. Soc. ital. Biol. sper.*, 1952, **28**, 445.
7. de Berardinis, E., *Rass. ital. Ottol.*, 1953, **22**, 345.
8. Karli, P., *Compt. rend. Soc. biol.*, 1952, **146**, 1770.
9. Karli, P., *Ophthalmologica*, 1954, **128**, 137.
10. Babel, J., and Ziv, B., *Ophthalmologica*, 1956, **132**, 65.
11. Rabinovitch, M., Thesis, Faculdade de Medicina de S. Paulo, 1953.
12. Noell, W. K., *Am. J. Ophth.*, 1955, **40**, No. 5, part 2, 60.
13. Dalton, A. J., *Anat. Rec.*, 1955, **121**, 281.
14. Trujillo-Cenoz, O., *Z. Zellforsch.*, 1957, **46**, 272.
15. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 319.
16. De Robertis, E., and Lasansky, A., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 743.
17. Yang, W. C., *Science*, 1957, **125**, 1087.
18. Guzmán Barron, E. S., *Advances Enzymol.*, 1951, **11**, 201.
19. Sorsby, A., Newhouse J. P., and Lucas, D. R., *Brit. J. Ophth.*, 1957, **41**, 309.

20. Wald, G., and Brown, P. K., *J. Gen. Physiol.*, 1952, **35**, 797.
21. Crane, R. K., and Ball, E. G., *J. Biol. Chem.*, 1951, **188**, 819.
22. Chinn, H. I., and Noell, W. K., United States Air Force School of Aviation Medicine, Project No. 21-23-011, Report No. 1, 1951.
23. Schimke, R. T., *Fed. Proc.*, 1957, **16**, 1428.
24. De Robertis, E., and Franchi, C. M., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 307.
25. Hodge, A. J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 221.
26. De Robertis, E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 209.
27. De Robertis, E., Gerschenfeld, H. M., and Wald, F., *Anat. Rec.*, 1958, **130**, 292.
28. Policard, A., Collet, A., and Pregermain, S., *Compt. rend. Acad. sc.*, 1957, **244**, 2458.
29. Fawcett, D. W., and Ito, S., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 135.

EXPLANATION OF PLATES

Legends

<i>dm</i> , dense material	<i>psc</i> , postsynaptic cytoplasm
<i>dmi</i> , degenerating mitochondria	<i>Rn</i> , rod nucleus
<i>DRIS</i> , dense rod inner segment	<i>rs</i> , rod sacs
<i>DROS</i> , degenerating rod outer segment	<i>Rs</i> , rod spherule
<i>drs</i> , degenerating rod sacs	<i>sm</i> , synaptic membrane
<i>dsm</i> , degenerating surface membrane	<i>sv</i> , synaptic vesicles
<i>dsy</i> , degenerating synaptic membrane	<i>v</i> , vesicles
<i>dsv</i> , degenerating synaptic vesicles	<i>vmi</i> , vacuolized mitochondria
<i>G</i> , Golgi	<i>VRIS</i> , vacuolized rod inner segment
<i>mi</i> , mitochondria	<i>vs</i> , vacuolized rod sac
<i>myf</i> , myelin figure	

PLATE 102

FIG. 1. Electron micrograph of the outer segment of a rod cell in a normal (control) rabbit. Observe the regular stacking of the rod sacs formed by double membranes. Many osmium granules are dispersed throughout the entire segment. For a discussion of these artifacts see reference 16. $\times 46500$.

FIG. 2. Rod outer segment of a rabbit, 6 hours after a single injection of 20 mg. of iodoacetate per kg. body weight. Observe the intense degenerative changes with vacuolization and lysis of the rod sacs. The region indicated with arrows shows a complete destruction. The surface membrane shows some discontinuities. $\times 30000$.

(Lasansky and De Robertis: Submicroscopic changes in visual cells)

PLATE 103

FIG. 3. Rod inner segments of a rabbit, 6 hours after a single injection of 20 mg. of iodoacetate per kg. body weight. The inner segment on the left shows a marked vacuolization of the matrix, the endoplasmic reticulum, and the Golgi complex. The mitochondria of the ellipsoid show fragmentation into smaller bodies (*dmi*). The inner segment on the right shows an increased density of the matrix with little vacuolization (*v*). $\times 35000$.

PLATE 104

FIG. 4. Similar treatment as in Fig. 3. Portion of a rod inner segment with large vacuoles in the matrix (*v*) and inside the mitochondria (*vmi*). $\times 93000$.

FIG. 5. Similar treatment as in Fig. 3. The main change consists in the increase in density with deposit of a dense granular material (*dm*) and some vacuolization of the matrix. $\times 93000$.

PLATE 105

FIG. 6. Rod cell of a rabbit, 18 hours after the second injection with 20 mg. of iodoacetate per kg. body weight. Alterations of the outer and inner segments are very intense. The rod sacs are vacuolized (*vr*s) and disintegrated. The surface membrane is mostly destroyed (*dsm*). The ellipsoid presents vacuolized and fragmented mitochondria (*dmi*) and there is a strong vacuolization of the cytoplasmic matrix. $\times$ 48000.

PLATE 106

FIG. 7. Electron micrograph of a cone synapse of a rabbit, 6 hours after a single injection of 20 mg. of iodoacetate per kg. body weight. The synaptic membrane (*sm*), the postsynaptic cytoplasm (*psc*), and some synaptic vesicles (*sv*) appear to be normal. In the central region of the pedicle there is an accumulation of granular material surrounded by concentric membranes having a honeycomb appearance (*myf*). These membranes apparently result from the fusion of vesicular material, including the synaptic vesicles. $\times 72000$.

PLATE 107

FIG. 8. Cone synapse of a rabbit treated as in Fig. 7. The process of membrane fusion has lead to the formation of concentric membranes typical of myelin figures (*myf*). $\times 46000$.

FIG. 9. Rod synapse of a rabbit, 18 hours after a second injection of 20 mg. of iodoacetate. The intense degeneration of the spherule is indicated by the lysis of most of the synaptic vesicles (*dsv*) and the destruction of the synaptic membrane (*dsv*). $\times 46000$.