and of parts of the cell membrane[13], require a larger energy expenditure, which could in turn induce morphological changes such as the appearance of granular aggregations in the energy producing mitochondria. This is also suggested by the large number of mitochondria containing aggregations as well as of aggregations per mitochondrion in the later stages after instillation when huge amounts of ferritin are absorbed and accumulated in the endothelial cells.

The observation reported here had, to the best of our knowledge, not been described before. Similar aggregations were not seen in our studies in mitochondria, either of alveolar macrophages or of alveolar epithelial cells, though these cells exhibit pronounced endocytotic activities[1]. This could indicate that the aggregations are specific for certain cell types (such as the lymphatic endothelial cells) under certain physiological conditions (i.e. high increase of the cell metabolism).

The precise nature of the granular aggregations in the mitochondria of lymphatic endothelial cells remains obscure. From this morphological study we can only conclude that they represent an accumulation of substances (ions, enzymes or other organic material) which play (or have played) a rôle in the increased respiratory activity of the mitochondria in response to an increased cell metabolism. The clustered appearance of the granules, which are often closely related to the inner or cristal membrane, may be explained by the occurrence of binding-places (possible on the membranes themselves) for these substances.

Résumé. Nous avons étudié les mitochondries dans les cellules endothéliales de lymphatiques pulmonaires chez des lapins nouveau-nés après instillation intratrachéale de ferritine ou de charbon. Les mitochondries des cellules qui ont endocyté de la ferritine et qui ont été fixées au glutaraldéhyde et au tétroxide d'osmium contiennent après coloration à l'acétate d'uranyle et au citrate de plomb des aggrégations de granules plus ou moins rondes (diamètre de 300 à 800 Å) qui d'après nous, n'ont pas encore été décrites auparavant. Quoique la nature précise de ces granules reste inconnue, il est possible qu'elles soient en relation avec une augmentation du métabolisme des cellules endothéliales, stimulées par l'endocytose et la digestion des particules de ferritine.

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Electron Cytochemical Demonstration of -SH Groups in the Synaptic Vesicles of Photoreceptor Cells with the Mixture of Zinc Iodide-Osmium Tetroxide

The mixture of zinc iodide-osmium tetroxide (ZIO) introduced by AKERT and SANDRI[1] in electron microscopy, stains totally different kinds of synaptic vesicles[1–5] and other subcellular components.

ZIO stains membranous structures similarly to conventional fixatives (glutaraldehyde, osmium tetroxide) in electron microscopy. However, electron opaque deposits, connected or not with membranes, may be seen in some structures. These electron opaque deposits characterize a positive ZIO reaction.

In the retina, MAILLET[6] observed with light microscope that ZIO stains the outer and inner plexiform layers. Electron microscopic studies made in our laboratory revealed a positive reaction in the synaptic vesicles of the outer[7,8] and inner[9] plexiform layers and in the rod outer segments in different species. Under certain conditions, the vacuolar system of photoreceptor cells was also reactive[10].

Synaptic vesicles do not show the same reactivity in both plexiform layers. When the fixation is made at 4 °C, only the vesicles of the outer plexiform layer react[7,8]. Their reactivity is variable and negative vesicles are frequently observed. On increasing the temperature (20 °C or more), a greater number of positive vesicles are observed, and the reaction is also seen in the synaptic vesicles of the inner plexiform layer[9].

The significance of ZIO reaction in synaptic vesicles has not yet been established. Synaptic vesicles with different transmitters are ZIO positive. Experiments made in vitro showed that many substances react with ZIO giving a black precipitate. Among them amino acids with –SH groups were amongst the most reactive. This fact, and other considerations[8], led us to think that –SH groups could be responsible of ZIO reaction. To test this hypothesis the effect of –SH reagents on ZIO reaction was studied in the synaptic vesicles of the photoreceptor cells of the rat.

Retinas from adult Wistar rats were quickly dissected after decapitation and incubated in toto at room temperature according to one of the following schedules: 1. in phosphate buffer 0.1 *M*, pH 6.9 for 30 min; 2. in 5 m*M* dithioerythritol (DTE) in the same buffer for 30 min; 3. in 0.1 *M* N-ethyl-maleimide (NEM) in the same buffer for 30 min; 4. incubation for 30 min in medium 2, washing for 5 min in the buffer, incubation for 30 min in medium 3.

After incubation the retinas were washed for 5 min in the phosphate buffer and a few seconds in distilled water to eliminate phosphates and fixed in ZIO at 4 °C for 2 h. After 10 min of fixation the retinas were teased in small blocks in a drop of ZIO. Tissue blocks were prepared for electron microscopy after fixation. Dehydration was done in ethanol and embedding in Epon 812. Ultrathin sections were stained with lead citrate and studied in a Siemens Elmiskop 1 electron microscope. ZIO was prepared as previously described[11].

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In another series of experiments, 0.32 *M* sucrose was added to the incubation media to improve the preservation of the general structure of photoreceptor cells which is affected by –SH reagents. Although sucrose seems to affect ZIO reaction in other structures (unpublished observations); the results of the experiments were essentially similar with or without sucrose in the synaptic vesicles of photoreceptor cells. Synaptic vesicles appeared more tightly packed in the nerve endings when sucrose was added. Phosphate buffer pH 6.9 was chosen because, at pH values higher than 7, significant hydrolysis of NEM itself takes place, and below pH 6 the reaction with thiols becomes too slow[12].

It was observed that after incubation in medium 1 (Figure 1) about 30% of the vesicles were total or partially electron opaque. When DTE, a –S–S-reducing agent, was added to the incubation medium, the proportion of reactive vesicles increased considerably, being about 60%, and a fine electron opaque precipitate could be observed in the other vesicles (Figure 2). After incubation in medium 3 (Figure 3) in which NEM, a –SH blocking agent, was incorporated, ZIO reaction was negative in the synaptic vesicles (compare with Figure 1). If the incubation in medium 2 (with DTE) was followed by incubation in medium 3 (with NEM) ZIO reaction (Figure 4) was almost totally negative. Exceptionally positive synaptic vesicles could be observed. Compare Figure 4 with Figure 2.

These results give support to the hypothesis that ZIO mixture, at 4 °C, under the conditions used, reacts with –SH groups in synaptic vesicles of photoreceptor cells, since after treatment with DTE the reactivity of the vesicles is increased and the reaction is blocked by NEM in the controls and in the retinas previously treated with DTE.

According to PEARSE[13], tissue –SH groups are entirely prevented from reacting in histological tests by prior

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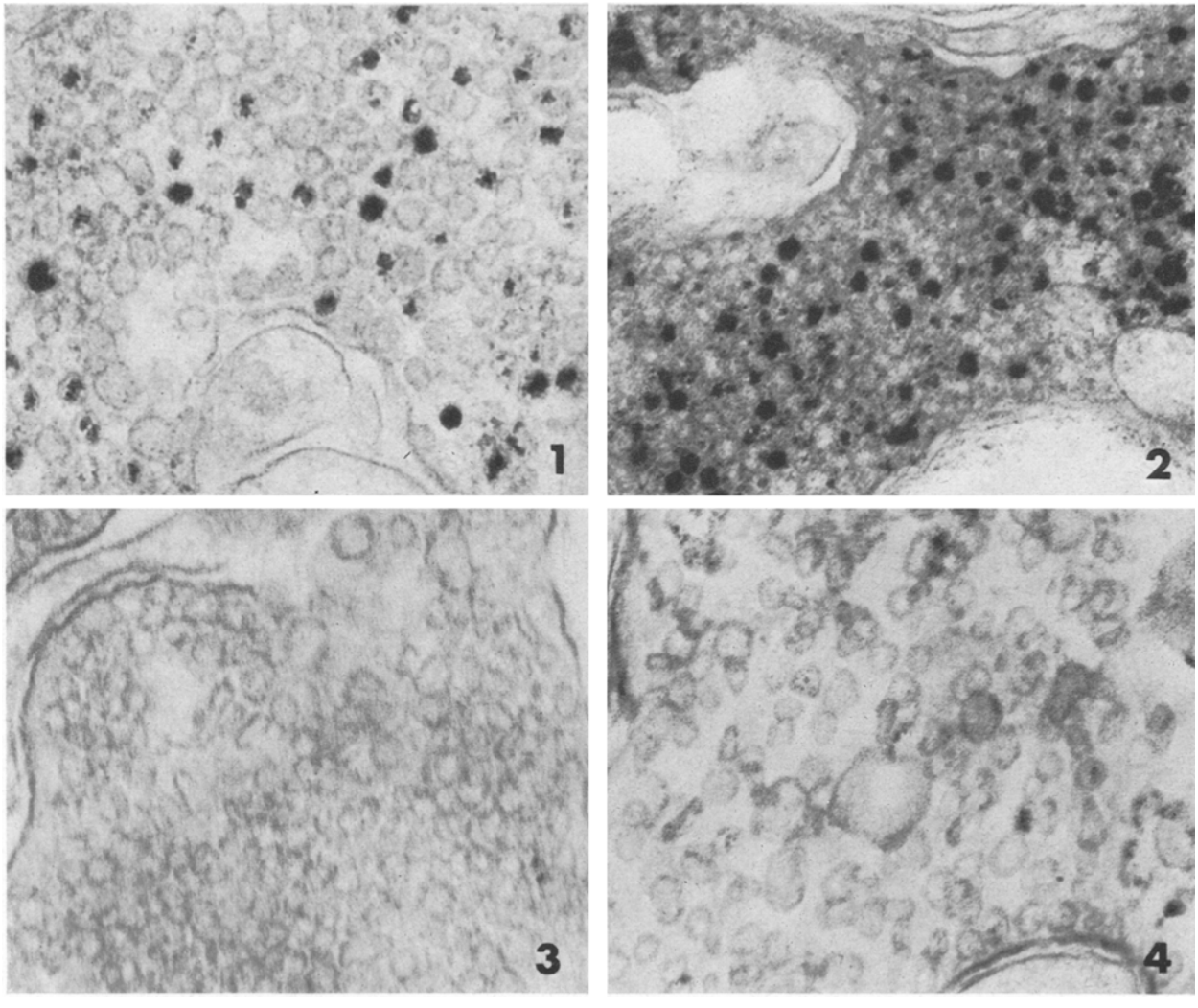

Figs. 1–4. Synaptic vesicles of photoreceptor cells of the rat stained with ZIO at 4 °C for 2 h. 1. After incubation in phosphate buffer 0.1 *M*, pH 6.9, with 0.32 *M* sucrose. A 30% of the vesicles are partial or totally electron opaque. ×120,000. 2. After incubation in 5 m*M* DTE in the same medium as Figure 1. The number of elctron opaque synaptic vesicles increased to 60%. A fine precipitate may be observed in the other vesicles. ×120,000. 3. After incubation in 0.1 *M* NEM in the same medium as Figure 1. All the synaptic vesicles appeared electron lucent. ×120,000. 4. After incubation in 5 m*M* DTE in phosphate buffer 0.1 *M*, pH 6.9, followed by incubation in 0.1 *M* NEM in the same buffer. Almost all the synaptic vesicles are electron lucent. Compare with Figure 3. ×120,000.

treatment with NEM. Although it has been shown that NEM can react with other amino acids in high concentration, it is unlikely that such reactions would occur at appreciable rates under the conditions used in this work[12].

The growing reactivity of the synaptic vesicles when the temperature is increasing, could be explained by the appearance of new –SH groups by rupture of –S–S-bridges.

Experiments which are in progress in our laboratory show that ZIO may reveal –SH groups in other sites such as the synaptic vesicles of pineal nerves[14] and the outer segments of rods. Our results seem to indicate that ZIO may be a useful tool to investigate –SH groups in tissues. However, the fact that ZIO reacts with many substances, makes a special study of each particular case necessary[15].

Resumen. Se demuestra, en un estudio in vitro, que el ditioeritritol (DTE), agente reductor de grupos –S–S- aumenta, la reactividad de las vesículas sinápticas de las células fotorreceptoras de la rata para la mezcla de yoduro de zinc-tetroxido de osmio (ZIO), a 4 °C durante 2 h, mientras que la N-etil-maleimida, bloqueante de grupos –SH, bloquea la reacción en los testigos y en los tratados previamente con DTE. Se concluye que ZIO a 4 °C durante 2 h revela grupos –SH en las vesiculas sinápticas estudiadas.

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Induzierte Strahlenresistenz bei Zellkulturen des Chinesischen Hamsters: Entwicklung und Art der Resistenz

Induced Radiation Resistance in Cell Cultures of Chinese Hamster Cells: Induction and Manner of Resistance

Für verschiedene biophysikalische Untersuchungen sind Zell-Linien unterschiedlicher Strahlensensibilität nützlich, wie z.B. für die Analyse der biologischen Wirkung einer neuen Strahlenart und von Reparaturphänomenen. Vor allem im Hinblick auf vergleichende Untersuchungen mit hochenergetischen Betatronstrahlen und Pionen entwickelten wir aus einem Basisstamm von Dauerkulturen embryonaler fibroblastenähnlicher Zellen des Chinesischen Hamsters eine gegenüber 200 keV-Röntgenstrahlen resistentere Linie, wobei uns 3 Fragen interessierten: 1. Welche Methode der Resistenzinduktion ist für unser Zellmaterial geeignet? Zellen in vitro können genetisch heterogen sein[1,2], so dass unter Umständen bereits eine unterschiedliche Strahlensensibilität für verschiedene Zellunterpopulationen besteht. Trifft dies zu, dann wäre Selektion eine geeignete Methode zur Erreichung einer strahlenresistenteren Linie[3]. Ist die Zellpopulation in bezug auf Strahlensensibilität relativ homogen, dann sollte versucht werden, durch Mutationsinduktion resistentere Zellen zu erzeugen, und diese sekundär einem Selektionsdruck zu unterwerfen[4]. 2. Was für eine Resistenzart entwickelt sich? Es kommen z.B. folgende Resistenztypen in Frage: Zellen mit vorwiegender Resistenz für niedere Dosen und Zellen mit Resistenz für höhere Dosen[4]. 3. Besteht eine Beziehung zwischen Resistenz und zellulärer Reparaturleistung? SHAEFFER und MERZ[5] sowie LITTLE et al.[6] fanden bei verschiedenen Zell-Linien keine Beziehung zwischen Strahlensensibilität und Erholungsphänomenen.

Material und Methoden. Zellmaterial und Aufzucht: Wir verwendeten Dauerkulturen embryonaler fibroblastenähnlicher Zellen des Chinesischen Hamsters, kultiviert als Monolayerkultur in Eagle's Basalmedium (Gibco G-13) and 20% fötalem Rinderserum sowie Zusatz von Antibiotika-Mischung (1%); pro Woche wurde 2 mal subkultiviert mit 0,2% Trypsin. Die Zellviabilität, gemessen mit der Trypanblaumethode, betrug 95%. Für die Bestrahlung wurden die Zellen in T-30 Greiner Plastikkulturflaschen (Gr. 2) in Monolayerkultur gehalten, das Nährmedium wurde ersetzt durch phosphatgepufferte Salzlösung (PBS); das mediumfreie Intervall dauerte 2–4 h.

Bestrahlung: Die Bestrahlung mit Röntgenstrahlen erfolgte bei 20–22 °C mit horizontaler Lage der Kulturflaschen: 200 kV, 12 mA, 1mm Al + 0,25 mm Cu, 46 rd/min, Fokus-Objekt-Abstand: 46 cm.

Bestimmung der Zellinaktivierung: 2 d nach Kulturansatz wurden die Zellen bestrahlt, gleich anschliessend trypsinisiert und pro Versuch ca. 1500 Zellen in je 2 Greiner – Plastikpetrischalen (Gr. 60/15) – mit eingezeichnetem Zählnetz am Schalenboden – ausgeplattet. 1 d nach Zellansatz Bestimmung der Zellzahl pro Netz und Zugabe von gereinigtem (dest. Wasser, Aceton) Bacto Agar (0,5%), 0,3 ml/ml Medium. Nach 10 d wurden die Klone mit Methanol: Eisessig (3:1) fixiert (10 min), luftgetrocknet und mit 0,1% Toluidinblau gefärbt (30 min). Bestimmt wurden die Anzahl Klone mit Zellzahlen $\geqq$ 50 Zellen innerhalb des Zählnetzes, bezogen auf die ursprünglich angesetzte Zellzahl; die Werte wurden um die jeweilige «plating efficiency» korrigiert (für Se_1: 20,2 $\pm$ 4,9 und für NMH_3: 42,7 $\pm$ 13).

Studium von Erholungsphänomenen: Es wurden a) eine mögliche Reparatur von subletalen Schäden durch Dosisfraktionierung[5,8] geschätzt, b) die Reparatur möglicher potentiell letaler Schäden nach WINANS et al.[9] ermittelt und c) die beiden Erholungsarten beeinflusst,

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