The Effect of Short Term Exposures to 100% Oxygen on the Fine Structure of Proximal Convoluted Tubules

The effect of short term exposures to pure oxygen on a variety of rat tissues has been described. SCHAFFNER and FELIG[1] and FELIG[2] reported loss of glycogen and mitochondrial enlargements and pleiomorphism in hepatic cells following exposure to oxygen for 3 days at 258 mm Hg, 1 day at 760 mm Hg and 3 h at 2280 mm Hg. These changes were followed by increased numbers of autophagic vacuoles, polyribosomal clusters and mitochondrial cristae. After 2 weeks at 258 mm Hg the alterations described had begun to regress, although numerous mitochondrial myelin figures were apparent. By 90 days at 258 mm Hg the liver cells appeared essentially normal. MAUTNER[3] similarly reported that after exposure to oxygen at 760 mm Hg for 1 day kidney tissue exhibited mitochondrial changes. PHILPOTT et al.[4] described me mbrane vesiculation in tissues of rats breathing 100% oxygen at $^1/_3$, $^2/_3$ and 1 atmosphere pressure for periods of time up to 1 month. As HAUGAARD[5] has pointed out when the oxygen tension in the atmosphere is elevated there is an increase in the amount of cellular oxygen, accompanied by unrecognizable metabolic changes and adaptations. Since the cells of the kidney cortex have the highest oxygen consumption and the lowest respiratory quotient of any tissue in the body, it was expected that high oxygen tensions could be tolerated by this organ. This hypothesis was explored, using a defined diet[6] to eliminate any possible alteration under oxygen that may be due to unrecognizable metabolite deficiencies or excesses. Sprague-Dawley rats (47–58 g) were exposed to 100% oxygen at 600 mm Hg continuously as described by SHAW[7]. Animals exposed to oxygen had pair-fed, air-exposed mates. Ad libitum air animals were also maintained. Samples from the kidney cortex were obtained at 3 and 7 days, fixed in Millonig's phosphate-buffered, 1% osmium tetroxide (pH 7.2) for 2 h and embedded in Epon. Sections were stained with saturated, aqueous uranyl acetate followed by Reynolds' lead citrate and examined in a RCA EMU 3F electron microscope.

The proximal tubular cells of animals exposed to 100% oxygen at 600 mm Hg for 3 days were essentially normal ultrastructurally as were their pair-fed mates and the ad libitum controls. No differences in the frequency of individual cell organelles were noted between the groups which could not be accounted for by normal factors such as depth of the tubules in the cortex and relative distance from the glomerulus. Some slight alterations were noted in the oxygen-exposed animals and their air-exposed, pair-fed mates after 7 days. In the pair-fed air animals the membranous spaces within the mitochondria cristae, Golgi, smooth endoplasmic reticulum, paramembranous tubular system and nuclear envelope appeared to have more of a tendency to dilate than those spaces in the ad libitum air animals. The mitochondrial cristae of 7-day oxygen-exposed animal were definitely dilated (compare Figures 1 and 2). Mitochondrial matrix granules present decreased in frequency, size and electron density. Large deposits of smooth endoplasmic reticulum were also noted in this group. At 7 days a definite increase in the number of lipid bodies was observed, however, other cell organelle frequencies in the proximal convoluted tubules were about the same.

The fine structure of the proximal convoluted tubules of rat kidney appeared largely unaffected by short term exposures to 100% oxygen at close to atmospheric pressures. Since oxygen-exposure produces anorexia and/or depresses metabolism, thereby reducing food intake, the slight ultrastructural changes observed in the 7-day-air-animals which were pair fed to oxygen mates can probably be attributed to the limited diets. The more noticable changes observed in animals exposed to oxygen for 7 days can be considered an additive effect of lowered food intake and oxygen toxicity.

Data compiled by JORDAN et al.[8] suggested that oxygen exposure decreases total fatty acid synthesis in the kidney and reduces the catabolic rate with an increase in total tissue lipid. The increased lipid bodies observed at seven days in oxygen-exposed animals may therefore reflect this increase in total tissue lipids.

The proximal convoluted tubule cells actively and passively resorb more than 80% of the glomerular filtrate including lipids or lipoproteins. The energy for this is supplied principally by the oxidation of fatty acids by

Fig. 1. Mitochondria from 7 day ad libitum air-control animal are shown from the basal portion of a proximal convoluted tubule cell. × 35,000.

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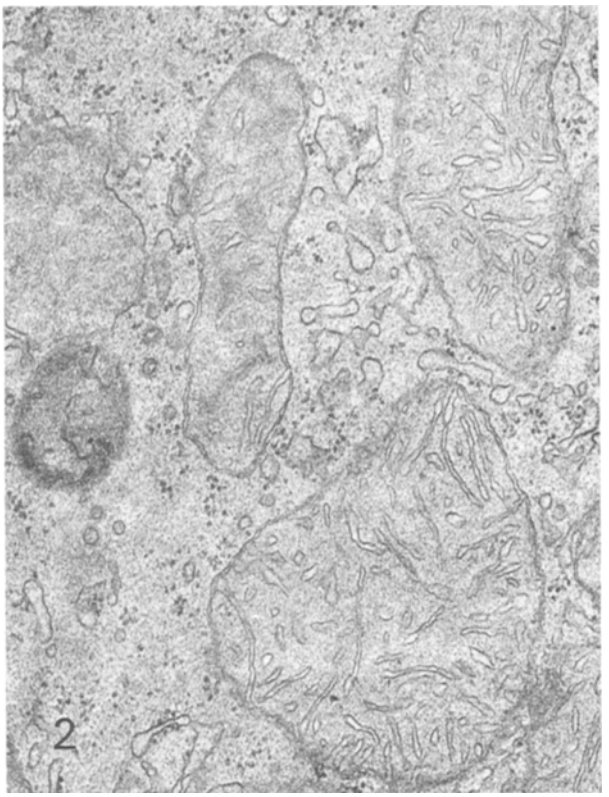

Fig. 2. Mitochondria from an animal exposed for 7 days to 100% oxygen at 600 mm Hg are shown. × 35,000.

the mitochondria, although alternative substrates including glucose, fructose, mannose, pyruvate and acetate can be utilized. JOANNY et al.[9] report an inhibition of tissue oxidative reactions and an increase of lipid peroxides in cerebral cortex slices exposed to hyperbaric oxygen. Altered mitochondrial metabolism caused by oxygen exposure at 600 mm Hg theoretically should account for the changes observed in mitochondrial ultrastructure[10].

Résumé. Des rats ont été maintenus dans une athmosphère d'oxygène pur sous une pression de 600 mm de mercure durant des périodes de 3 et 7 jours. Les observations faites au microscope électronique sur les tissus prélevés dans le rein ont montré qu'après 7 jours de traitement les mitochondries et les particules de corps gras ont subi dans la cellule d'importantes modifications. Après 3 jours seulement, on n'observait accun changement de l'ultrastructure.

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Monoamine-Containing Structures in the Nerve Cord of Some Representatives of Diptera (Insecta)

The fluorescence histochemical method developed by FALCK and HILLARP[1,2] has made possible the study of the cellular localization of some biogenic monoamines. The first positive results in certain representatives of the class of insects by this method were obtained by FRONTALI and NORBERG[3], PLOTNIKOVA and GOVYRIN[4], FRONTALI[5], KLEMM[6,7], CHANUSSOT et al.[8]. If there is no doubt about the presence of catecholamines in the brain and the stomatogastric nervous system in insects, the same cannot be said, for the time being, about the nerve cord ganglia. The experiments performed by the authors cited above have not yielded any answer to this question. That is why we have tried to augment our knowledge in this field. The histochemical demonstration of catecholamines was performed partly on whole mounts of the ventral nerve cords, partly on serial sections from lyophylized individuals.

Our observations were made on larvae of the same developmental stage – before the pupation – of 2 representatives of dipterous insects, i.e. *Simulium argyreatum* (Simuliidae) and *Ptychoptera contaminata* (Ptychopteridae), both from the suborder of Nematocera.

In the preparation of whole mounts of the ventral nerve cords for the relevant studies, we proceeded so as to observe the conditions recommended by MALMFORS[9]. The actual preparation of nerve cords was performed with the aid of a dissecting microscope and never lasted longer than 10 min. The isolated cords were melted onto dry glass slides and dried for 1 h in a vacuum over phosphorus pentoxide. The dried nerve cords were treated with dry formaldehyde gas (paraformaldehyde store at 70% humidity) for 1 h. After they had been mounted in liquid paraffin, they were evaluated in a fluorescence microscope with Schott BG 12 and OG 4 filters. The

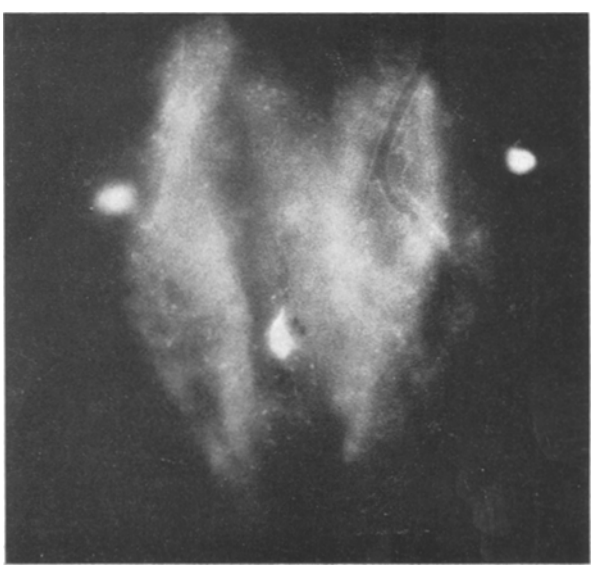

Fig. 1. 3 fluorescent regions in the first abdominal ganglion of the nerve cord of *P. contaminata*, a whole mount.