(−100°C), 5.87; Na_2O, 0.002; K_2O, 0.039; CaO, <0.007; and MgO, 0.004. Tabashir contains more water than is commonly found in opals (*5*) but smaller amounts of alkalis and alkaline earths.

The statement (*4*) that tabashir is silica gel led us to examine a pure silica gel in the electron microscope. The gel, which was produced by drying a solution of monosilicic acid at 80°C, had no detectable alkalis or alkaline earths. Grinding produced fragments which ranged in size from a few microns to several hundred angstroms and showed no internal structure. The featureless structure of the irregular fragments and the tapering edge of a larger fragment are shown in Fig. 1*f*. Replicas of fracture surfaces had a slight texture but were smooth by comparison with tabashir. Silica gel therefore differs from tabashir and is homogeneous to the limit of resolution of the microscope (10 Å).

We then compared tabashir with opal phytoliths, which we separated from oats by destroying organic matter with nitric and perchloric acids (*2*). Grinding and dispersing the phytoliths produced a heterogeneous mixture of small fragments. In the electron microscope many of these fragments were similar to tabashir, but the basic particles were rather larger and more irregular. There were in addition some larger, irregularly shaped fragments up to 1000 Å in size with a homogeneous appearance.

Although tabashir and opal phytoliths have rather similar physical properties (including specific gravity and index of refraction), opal phytoliths contain greater proportions of alkalis and alkaline earths (*2*). This difference in purity is reflected in a different reaction to heating; both forms crystallized to the low-temperature form of cristobalite, but in the opal phytoliths the crystallization occurred at 1050°C (*2*), 200°C below the crystallization temperature of tabashir.

Electron diffraction from tabashir, opal phytoliths, and silica gel gave only a diffuse ring corresponding to a lattice spacing of 4.0 Å. However, the carbon supporting film gives weak, diffuse rings at 2.0 Å and 1.1 Å, which could conceal any similar but weak diffraction from silica fragments. Although the patterns from opal phytoliths and silica gel were indistinguishable, the pattern from tabashir was always sharper than either of them. The x-ray diffraction patterns from the three varieties of silica differed slightly. Thus in addition to the spacing at 4.0 Å, tabashir gave three diffuse bands, opal phytoliths gave one (at 1.97 Å), and silica gel gave none.

Because the additional bands appeared in x-ray patterns of tabashir and opal phytoliths but not in electron-diffraction patterns, we can infer that they were not produced by a homogeneous structure of disordered or microcrystalline cristobalite. Electron diffraction is the more sensitive technique, but the x-ray diffraction as carried out here was more selective because generally the pattern was obtained from a single fragment a few microns in size. The very low intensity of the extra x-ray bands relative to that at 4.0 Å is therefore in keeping with the interpretation that they were produced from a small fraction of the sample rather than from homogeneous, long-range ordering or incipient crystallization.

Although tabashir, opal phytoliths, silica gel, and many opals of inorganic origin are amorphous, the two forms of opal of plant origin can be distinguished from the others in the electron microscope. Because the electron optical microstructure of tabashir differs so markedly from that of amorphous opal of inorganic origin, tabashir appears to deserve a special place among the varieties of opaline silica.

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Hyperbaric Oxygenation: The Eye as a Limiting Factor

Abstract. *In dogs exposed to 100 percent oxygen at 3 atmospheres absolute pressure for more than 4 hours, a characteristic retinal lesion, manifested as the cytoid-body change, occurs. The selectivity of this injury suggests that the eye may be used as a sensitive indicator and as a site for study of oxygen toxicity. The cytoid body, an entity of disputed genesis, was produced experimentally for the first time.*

In recent years, through collaboration between the engineer and the medical scientist, thresholds have been crossed freeing man for finite periods from his dependence on the earthly atmosphere. These advances, enabling man to probe into space, to extend his reaches undersea, and to use hyperbaric oxygenation in medicine, are dependent upon the establishment and maintenance of a life-sustaining milieu, independent of and isolated from the surrounding environment. Oxygen is a major component of this milieu. Yet, paradoxically, a crucial limiting factor in the exploitation of this advance is man's intolerance to oxygen at high ambient pressures or concentrations. In our studies the eye has been a consistent and early indicator of oxygen toxicity, and we have observed a new pathologic manifestation of this injurious effect.

Our data are part of a comprehensive study of the applications and limitations of hyperbaric oxygenation in medicine (*1*). Using standard Navy diving and decompression techniques (*2*), we placed mature, healthy mongrel dogs individually, without anesthesia and without restraint, in a 1-m^3 chamber and exposed them to 100 percent oxygen. Oxygen inflow was regulated at the inflow valve to maintain a pressure of 30 lb/in.^{2} gauge pressure, equivalent to approximately 3 atm absolute pressure, while the chamber was being vented at 0.014 m^3 per minute. Soda lime was used in the chamber to extract carbon dioxide. Each animal was subjected to a single exposure ranging from 210 to 362 minutes, regardless of adverse effects, which were predominantly manifested as seizures. These seizures developed at irregular intervals with abrupt onset and spontaneous cessation and in no instance led to paralytic sequelae or evidence of neurologic damage. For 6 to 13 days following exposure, the ani-

mals were observed for the presence of neurologic impairment. Then they were killed by a perfusion-fixation method modified from both Cammermeyer and Malm techniques (*3*), and subjected to pathologic study both by routine histologic methods and by techniques designed for studies of the nervous system (*4*).

The only change attributable to oxygen toxicity found in somatic tissues or in the central nervous system of these animals was a characteristic ocular lesion (Fig. 1). This lesion consisted of a segmental degeneration of axons in the nerve-fiber layer of the retina in the region encompassing and surrounding the optic-nerve head. When axons were stained the degenerated fibers were seen to terminate abruptly in clublike swellings or appeared as clusters of globular structures.

In hematoxylin-eosin stains, they frequently demonstrated a pseudonucleus and pseudocytoplasm. They appeared identical to cytoid bodies, the microscopic components of the "cotton wool spot," which is characteristic of retinal vascular disease in man (*5*). Although rare instances of necrosis of retinal ganglion cells were also observed, the cytoid-body change was a far more conspicuous feature and appeared to be a primary feature of the injury. This lesion was encountered in the eyes of 12 dogs subjected to a single hyperbaric exposure of 240 minutes or more; it was absent in two dogs, one exposed for 210 minutes and the other for 225 minutes. The lesion was not observed in 83 animals subjected to shorter periods of hyperbaric oxygenation, ranging from 45 to 120 minutes, in studies of the protective effect of hyperbaric oxygenation against sequelae of circulatory arrest. Since the lesion is so prominent when viewed microscopically, it is likely that its gross counterpart, the cotton wool spot, might be seen ophthalmoscopically, and therefore be a useful sign of oxygen toxicity. Gross recognition of the lesion is prerequisite for projected studies of ultrastructure. The discovery of this lesion was unexpected, however, and we have not yet had an opportunity to observe it in a living animal.

Ever since the etiology of retrolental fibroplasia was established, the sensitivity of the immature retina to the toxic action of oxygen has been generally recognized. Ashton and Pedler (*6*) demonstrated that ambient hyperoxia exerts a selective action upon the endothelium of developing retinal vessels but does not injure directly other retinal structures or affect immature cerebral or meningeal vessels. Noell (*7*) has demonstrated that this sensitivity exists also in the mature eye, but with the visual cells rather than the vascular bed as the target tissue. Exposure to 100 percent oxygen at 1 atm for more than 24 hours caused a depression of the electroretinogram (ERG).

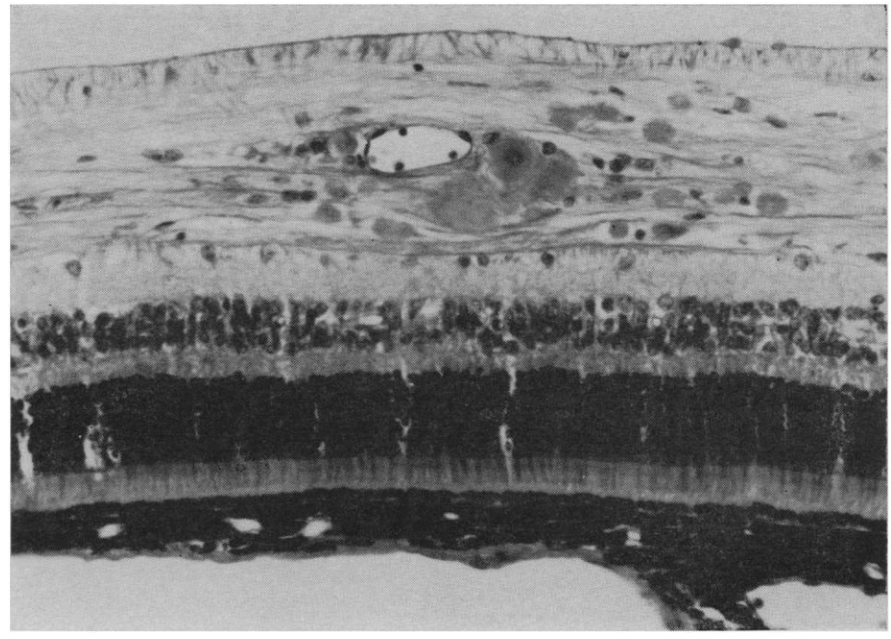

Fig. 1. Characteristic retinal lesion observed in dogs exposed to 100 percent oxygen at 3 atm absolute pressure for a period of more than 4 hours. Numerous globular bodies are visible at all levels of the nerve-fiber layer, particularly bordering retinal vein. A pseudonucleus characteristic of the cytoid body is present in the globule to the right of the vessel. The absence of changes in the visual cells is notable. Hematoxylin-eosin (× 180).

These changes occurred in the absence of other manifestations of oxygen toxicity. With hyperbaric oxygenation these alterations were both accelerated and intensified. Depression of the ERG was greatest after 3 to 5 hours exposure to 100 percent oxygen at 3 atm, and irreversible injury to visual cells was regularly recognized at 5 to 6½ hours and frequently after shorter exposures.

The significance of our observations for research in hyperbaric oxygenation resides in: (i) the demonstration of a selective retinal injury in the dog, the animal commonly used as the "proving ground" for physiologic and therapeutic procedures applicable to man in the cardiovascular field; and (ii) the development of the lesion at pressure levels commonly used in experimental studies of oxygen hyperbarism and after exposure times approximating the estimated safe thresholds for man.

Beyond these considerations, the production of this retinal lesion is of salient importance for investigative ophthalmology. It constitutes for the first time the experimental production of the cytoid body, a lesion whose nature and pathogenesis have been the subject of a century-old speculation (*5*). The progressive contraction of visual fields and impairment of central vision in man (*8*) after exposure to 100 percent oxygen at 3 atm for more than 4 hours may then be considered danger signals heralding the onset of irreversible retinal injury if exposure continues.

Death of visual cells, as described by Noell in the rabbit—an animal with an avascular retina—and the segmental degeneration of nerve fibers that we observed provide two different anatomical indices useful for further studies of the pathogenesis of oxygen toxicity. Although the strong vasoconstrictive reaction of the retina to oxygen (*9*) suggests the possibility of ischemic anoxia, it is difficult to accept anoxia as a likely causative mechanism. Increased oxygen transport in oxygen hyperbarism may more than compensate for reduced blood flow, since the retinal veins have an arterialized appearance and since hyperbaria prolongs ret-

inal function in retinal ischemia (*10*). However, a profound and prolonged vasospastic response to oxygen could produce the paradoxical situation of localized anoxia in an oxygen-rich environment.

On the other hand, injury due to the inhibition by oxygen of intracellular enzyme reactions could readily account both for the death of visual cells and for segmental lesions in their processes. Oxygen at elevated pressures is a potent toxin for living cells (*11*). Support for the concept that oxygen at high concentrations has a direct toxic action on the intact animal is found in the potentiating action of carbon dioxide upon the neurotoxic effects. Convulsive responses, paralysis, and death are all accelerated or increased in incidence by the addition of this carbon dioxide (*12*).

Preliminary studies in our laboratories (*1*) indicate that the eye lesion due to hyperoxia may be prevented by the use of 98 percent oxygen and 2 percent carbon dioxide at 3 atm. However, with this respiratory gas mixture, there was a shift in the locus of toxic action of oxygen from the eye to the central nervous system which was manifested clinically by overt neurologic deficits and histologically as selective neuronal necrosis. Two separate mechanisms may be operative; the increased vascular resistance induced by hyperoxia may, on the one hand, result in ischemic retinal injury, and on the other hand, protect the central nervous system from the histotoxic effects of hyperoxia. There is much speculation regarding the pathogenesis of oxygen toxicity. If these preliminary findings prove correct, the therapy of oxygen poisoning may be more problematic than hitherto thought.

The perils to the eye posed by oxygen at high pressure are of particular significance to hyperbaric medicine and to undersea ventures. Since, however, prolonged exposure to oxygen at a partial pressure of over 350 mm-Hg is regularly attended by toxic manifestations (*13*) our man-in-space program carries this hazard, too (*14*).

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Auxin and Kinetin Interaction in Apical Dominance

Abstract. *The effects of auxin on the inhibition of lateral buds in decapitated bean plants are enhanced if kinetin is applied together with auxin. The uptake of ^{14}C-indoleacetic acid by the stumps of decapitated plants is increased in the presence of kinetin and leads to extensive transport of ^{14}C-indoleacetic acid in the stems. The increased bud inhibition resulting when auxin and kinetin are applied together may be due to greater amounts of auxin reaching the buds, but an alternative explanation is that metabolites are directed from the buds to the point of hormone application.*

Until recently it was thought that the correlative inhibition of lateral buds is regulated by a single hormone, auxin, produced in the shoot apical region and moving downwards in the stem, where it directly inhibits the growth of the buds. It is now becoming apparent that in bud inhibition, as in other growth phenomena, there is an interaction between the three types of hormones, auxin, kinin, and gibberellin.

Sachs and Thimann (*1*) showed that if auxin is applied to the stump of decapitated pea seedlings, the resulting inhibition of the lateral buds is released if kinetin is applied directly to the lateral buds. Jacobs and Case (*2*) presented evidence that auxin and gibberellin may interact in apical dominance. They found that if gibberellic acid (GA) and indoleacetic acid (IAA) were applied together to decapitated pea seedlings, the buds remained inhibited for a longer period than if IAA alone was applied. They demonstrated that the transport of ^{14}C-labeled IAA in decapitated pea stems is enhanced when GA is also applied, and they suggested that the observed increased apical dominance can be ascribed to the greater amounts of IAA reaching the buds when IAA and GA are applied together. We have observed similar interaction between kinetin (6-furfurylaminopurine) and IAA in apical dominance, but a different interpretation of the results from that of Jacobs and Case is suggested.

Experiments were conducted with seedlings of French bean (*Phaseolus vulgaris*, cv. 'Canadian Wonder') which were grown in "John Innes" potting compost in boxes in a heated greenhouse. The seedlings were grown for 2 weeks and were then decapitated 5 cm above the node of the paired primary leaves. Preparations of IAA and kinetin in lanolin, each at a final concentration of 0.1 percent, were applied alone and in combination to the decapitated internodes. Equal quantities of lanolin, contained in gelatin capsules (about 1 ml), were applied to each plant; in this way the lanolin was applied to the cut surface and to the uppermost 2 mm of the sides of the stump. Measurements were made daily of the lengths of the basal internodes of the buds in the axils of the primary leaves (Fig. 1). Treatment with both IAA and IAA plus kinetin resulted in inhibition of bud growth up to the 5th day. Thereafter the buds of the IAA-treated plants began to grow out slowly, whereas those treated with IAA and kinetin remained inhibited, so that by the 14th day the two treatments had resulted in greatly different growth. The buds of the lanolin controls and of the kinetin-treated plants began to grow out after the 1st day, and they continued