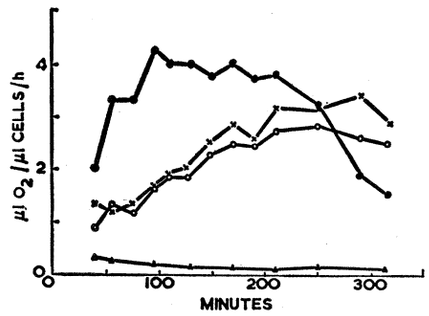

Fig. 1. Toxic effects of polyethylene glycol 1540 on glucose-induced respiration of *Chlorella pyrenoidosa*. (●———●) control, —0.4 atm; (×———×) mannitol, —10 atm; (○———○) PG1540, —10 atm purified both 14 months earlier and repurified prior to the test, by passage twice through exchange columns; (▲———▲) PG1540, —10 atm; purified only 14 months earlier. Storage of the PG1540 stock solutions was at room temperature.

Table 2. Respiration of potato disks as affected by "purified" polyethylene glycol 1540 stored at room temperature or at 1°C.

Treatment	Water potential (atm)	O_2 (μl/5 disks per hr)
Control	0	44
PG1540, room temperature, 6 months	−10	9.3
PG1540, 1°C, 6 months	−10	34
Freshly purified PG1540	−10	36

development of inhibitor in PG200, during a period of 12 months' storage at room temperature. In conclusion, many experiments using polyethylene glycols to impose a water stress on living organisms must remain suspect, unless details on purification and storage are also reported.

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Rat Kidney Centrioles: Vitamin E Intake and Oxygen Exposure

Abstract. *The incidence and morphology of centrioles in the proximal convoluted tubules of the rat kidney are altered by the dietary content of vitamin E, but not vitamin A, and by an increase in the pressure of oxygen. Increases in the number of centrioles in rats depleted of vitamin E and in those exposed to oxygen suggest that an oxidative mechanism is involved in the centriole alterations. The known antisterility action of vitamin E may thus be related to the direct protection of the mitotic apparatus.*

The first known manifestation of deficiency in vitamin E is reproductive insufficiency (*1*). The effects of vitamin E on reproduction cannot be reversed by selenite, cystine, or nonspecific antioxidants but are ameliorated by small amounts of methylene blue and similar antioxidants of appropriate redox potential and biological availability. The effects of deficiency in vitamin E upon the reproductive capacity of the rat are so well known that they serve as the bioassay method for this vitamin (*1*). The direct effects of vitamin E deficiency upon cellular function as it influences reproduction are not known. We have noted that the prevalence of centrioles in the rat kidney is influenced by amounts of vitamin E in the diet and by exposure to increased oxygen tensions.

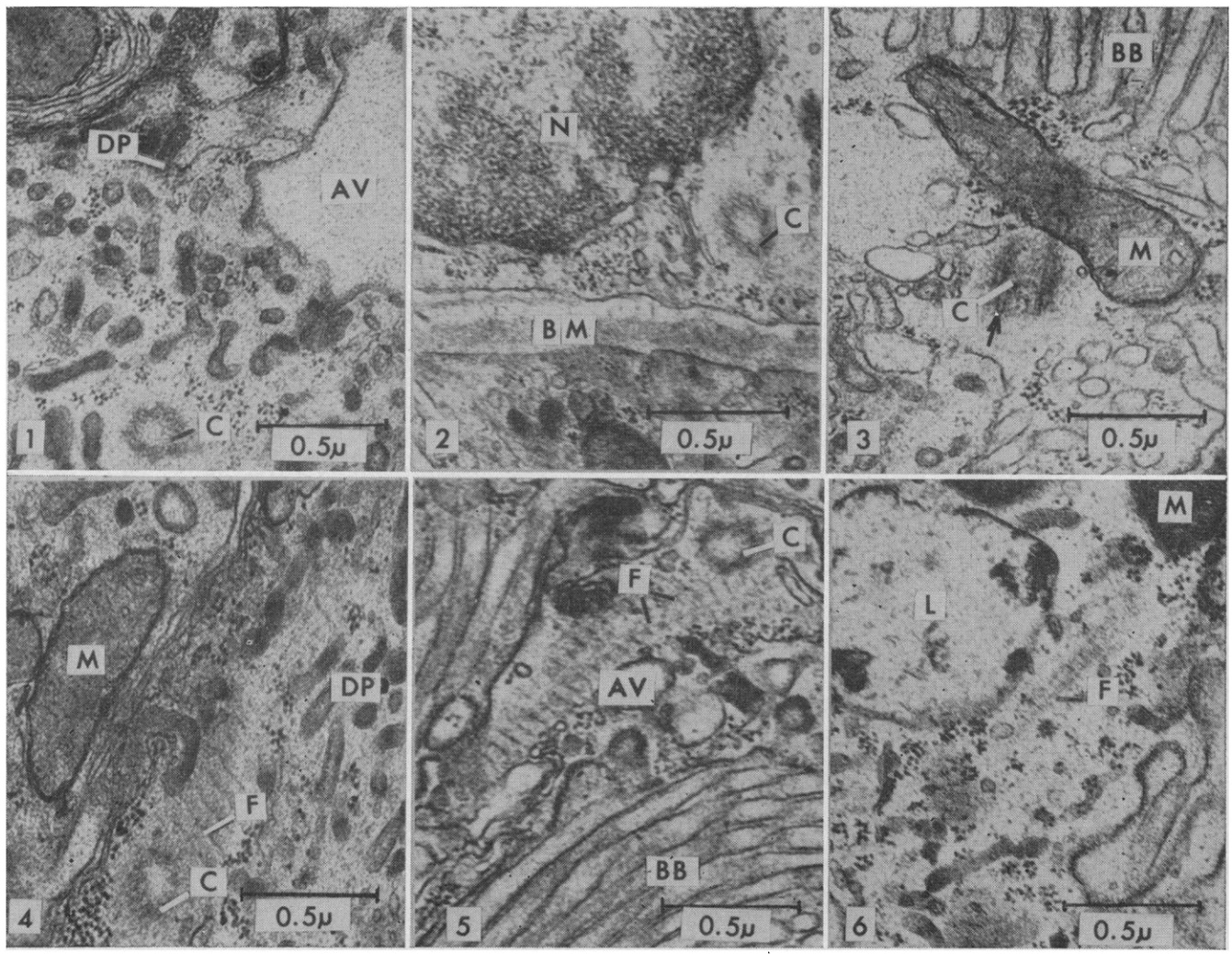
The proximal convoluted tubule cells of Sprague-Dawley rats exposed to a 100-percent oxygen atmosphere at 600 mm-Hg, or ambient air, were examined for the presence of centrioles. Animals were exposed to the oxygen environment for 3, 7, or 35 days (*2*). They were fed chemically defined diets (*3*) complete in all known nutrients. Vitamins were supplied at three times the minimum amounts, except for vitamins A and E. The vitamin A contents were 0, 2000, or 20,000 international units per kilogram of ration in combination with 0, 66.5, or 665 mg of vitamin E (per kilogram) as α-tocopherol acetate. In addition, some animals were kept in air and fed commercial laboratory ration (Purina). The specific diet fed to the experimental animals is indicated in the figures. Slices from the cortex of the kidneys were minced in either 3-percent phosphate-buffered glutaraldehyde (*p*H 7.2) or Millonig's 1-percent osmium tetroxide (*4*). Tissues fixed in glutaraldehyde were subsequently fixed in osmium; they were dehydrated in a graded ethanol series and embedded in Epon 812. Sections were stained in saturated, aqueous uranyl acetate and then in Reynolds' lead citrate (*5*).

Centrioles and pericentriolar bodies in the proximal convoluted tubule cells of the rat kidney were most frequently observed in the apical region of the cell, either immediately at the base of the microvilli of the brush border or among the dense tubular profiles and vesicles associated with the microvilli (Fig. 1). Centrioles without associated filaments were also sometimes noted in the juxtanuclear basal regions of the cell just above the basement membrane (Fig. 2). The centrioles, similar in structure to those described in other works (*6*), consisted (in cross section) of nine groups of triplet filaments. Longitudinal sections show that they are hollow cylinders approximately 200 nm in diameter. The sides of the cylinder appear to be formed of two double-component membranes, each of which sometimes appears scalloped or composed of small vesicles. The centrioles are open at one end and closed at the other by an invaginated inner core which appears to be directly continuous with the inner membrane of the cylinder (Fig. 3, arrow). A smaller vesicle can be seen in the center of this invagination. Thin filament-like extensions which apparently are connected to the fine web comprising the cytoplasmic ground substance radiate from the sides of the centriole in both oblique and longitudinal sections (Figs. 1 and 3). Also associated with centrioles near the brush border and sometimes apparently continuous with their surface are filamentous bodies (Figs. 4 and 5) which consist of a series of parallel periodic filaments at right angles to the longitudinal axis of the centriole. These filaments occur singly (Fig. 4) or in groups (Fig. 5). In most instances, the parallel bands lack interconnections; however, there are occasional cross-linking filaments (Fig. 6). Those filamentous bodies with longitudinal and transverse filaments also have major and minor bands. Only one type of pericentriolar filamentous body occurs with a single centriole at any one time, and either type of filamentous body occurs in all experimental groups.

Whether the differences in morphology of the filaments represent two different structures or indicate different physiological states of the cell could not be determined. The transverse filaments appear as wide as 200 nm (Fig. 4) but average about 700 Å.

A sampling of comparable numbers of sections from the same distal region in the proximal tubule cells revealed the following trends in the occurrence of centrioles and pericentriolar filaments. Animals on defined diets with no added source of vitamin E had more than twice as many centrioles and pericentriolar bodies as any other dietary group in air had. (Five centrioles were observed in animals deficient in vitamin E for every two centrioles observed in those with sufficient vitamin E.) The next highest incidence of centrioles, found in animals on a commercially prepared diet that contained minimum quantities of vitamin E, was approximately 33 percent more than in those animals with adequate amounts of vitamin E. Further, the frequency of centrioles and associated filaments in the proximal convoluted tubule cells of all rats exposed to 100 percent oxygen was approximately double the frequency in the paired animals exposed to air. Serial sections occasionally revealed some cells with more than one pair of centrioles and pericentriolar filaments. The filaments of animals exposed to air exhibited a periodicity of approximately 700 Å, whereas in animals exposed to oxygen the periodicity varied from 560 to less than 500 Å.

While the morphology of centrioles and pericentriolar filaments of the rat kidney has not been previously described, centrioles and pericentriolar bodies have been reported in normal and pathologic rabbit glomeruli (*7*). In a study of mitosis in the kidney tubules of young rats, Jokelainen (*8*)



Figs. 1–6. Cross section of kidney centriole (*C*) from animal exposed to air and fed a diet containing 66.5 mg of vitamin E per kilogram. *AV*, apical vacuole; *DP*, dense profile. Fig. 2. Basally located centriole (*C*) from rat exposed to oxygen for 3 days, fed a diet containing 66.5 mg of vitamin E per kilogram. *BM*, basement membrane; *N*, nucleus. Fig. 3. Longitudinal section of centriole (*C*) from rat exposed to ambient air for 35 days. Diet had no added source of vitamin E. *BB*, brush border; *M*, mitochondria. Fig. 4. Pericentriolar filaments (*F*) and centriole (*C*) from the proximal convoluted tubule of rat exposed to air and on diet containing no added source of vitamin E. Periodicity of filaments, approximately 700 Å. *DP*, dense profile; *M*, mitochondria. Fig. 5. Rat exposed to oxygen and on diet with no added source of vitamin E for 35 days. Periodicity of filaments, approximately 560 Å. *AV*, apical vacuole; *BB*, brush border; *C*, centriole; *F*, pericentriolar filaments. Fig. 6. Pericentriolar filament with major and minor bands. Periodicities, approximately 500 Å (major band), Å (minor band). Rat exposed to oxygen and depleted of vitamin E. *F*, Pericentriolar filaments; *L*, lysosome; *M*, mitochondria.

did not observe pericentriolar bodies associated with the centriole of metaphase cells; he suggested that pericentriolar satellites apparent at interphase are not present at metaphase. Cilia associated with centrioles in the rat kidney appear to be highly infrequent in normal mammalian metanephros (*9*). Occasional cilia are observed in human mesonephros, but they are frequent in the pronephros; this suggests that cilia are an evolutionary remnant in the proximal tubules (*10*). Serial sections failed to indicate that the centrioles that we observed were associated with cilia, although the position of the centrioles close to the base of the microvilli and the presence of pericentriolar filaments resembling striated rootlets do not preclude this possibility. Cilia can be induced in interphase fibroblasts by the mitotic inhibitor colcemid (*11*). Because the centrioles in our study are apparently from cells arrested at interphase and occur in proximal tubule cells having a ciliary background, the pericentriolar filaments may have been induced by the combined stresses and could possibly differentiate into cilia.

The alterations of centrioles may also be related to their mitotic function. The frequency of centrioles was lowest in animals raised in air and receiving a chemically defined diet that contained adequate or greater quantities of vitamin E. Apparently, the quantity of vitamin A did not influence the number of centrioles. If it is assumed that centrioles will be observed only infrequently in normal mitosis, then the increased numbers of centrioles observed in animals depleted of vitamin E and exposed to oxygen suggest a dysfunction in the formation of the mitotic apparatus. Increased numbers of centrioles could result from an increased rate of mitosis or from inhibition of mitosis after centriole proliferation but before spindle formation. The accumulation of centrioles in animals exposed to air and depleted of vitamin E and in those exposed to oxygen suggests that an oxidative function is involved. Ample evidence of the antioxidant role of vitamin E exists (*12*). Sulfur amino acids are known to be important in the time sequence of the symptoms of muscular dystrophy associated with deficiency of vitamin E (*13*), and they are known to be easily oxidized by molecular oxygen or lipid peroxides (*12*). Molecular oxygen inactivates enzymes containing SH groups as well (*14*). Centrioles contain concentrations of SH groups; the maintenance of the reduced state of these SH groups appears necessary for the completion of mitosis (*15*). Soluble thiols (perhaps glutathione) decrease up to metaphase and then increase as cell division proceeds. Increased tensions of oxygen decrease (i) the concentrations of reduced glutathione in the blood (*16*), (ii) tissue protein SH groups, and (iii) nonprotein SH groups (*3*). The dysfunction of the centrioles may involve oxidation of cellular lipids which accumulate, or it may involve oxidation of SH groups. Vitamin E may function to protect SH groups directly or indirectly through an antioxidant chain. The pericentriolar bodies may be associated only with interphase, and the properties of vitamin E that alleviate sterility may be involved in the direct protection of the mitotic apparatus.

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Murine Hepatitis Virus: Effect on Liver RNA

Abstract. *After infection of mice with hepatitis virus MHV3, the RNA in the liver undergoes changes. The fraction extracted with phenol at 0°C does not alter. However, the fraction extracted with hot phenol at elevated* p*H (60°C,* p*H 8.3) shows a 16*S *peak on sucrose-density-gradient centrifugation. This fraction shows actinomycin D–resistant incorporation of* C^{14}*-orotic acid in infected but not in control livers—possible evidence of the RNA nature of MHV3.*

Direct evidence on the genetic material of hepatitis viruses has been difficult to obtain. In studies of murine hepatitis virus MHV3, elevation of serum enzymes (*1*), alteration in tissue culture morphology (*2*), changes in oxidative enzymes (*3*), lysosomes (*4*), and electron microscopic appearance (*5*) have been described. Actinomycin D and fluorodeoxyuridine have no effect on the course of the infection (*6*). From these findings, it may be tentatively concluded that MHV3 is an RNA virus, is independent of DNA-directed synthesis of RNA or DNA replication, and requires the induction of an RNA-RNA polymerase (*7*). More direct evidence of the RNA nature of the virus requires demonstration of alteration of RNA metabolism in infected livers, this alteration being insensitive to actinomycin D.

The MHV3 virus obtained from the Rockefeller strain was inoculated into 21-day-old mice (white inbred Swiss strain). The strain was propagated by (i) homogenization of livers (10 percent weight to volume) infected for 48 hours, (ii) centrifugation at 2000*g*, and (iii) intraperitoneal injection of the supernatant. The supernatant stock was stored at −20°C. The course of the infection was determined by gross and microscopic examination of the liver and also by determination of serum lactate dehydrogenase and alanine aminotransferase (E.C. 2.6.1.2.). Seven hours before they were killed, infected and control mice received intraperitoneal injections of saline or of saline containing actinomycin D (100 μg per 100 g of body weight); 4 hours later, 20 μc per 100 g of body weight of C^{14}-orotic acid was also injected (specific activity, 5 mc per millimole). After the mice were killed, the liver was