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27 November 1967

Respiratory Inhibition in Chlorella Produced by "Purified" Polyethylene Glycol 1540

Abstract. *Polyethylene glycol 1540, added to culture solution in amounts sufficient to reduce the water potential to −10 atmospheres, inhibited respiration in* Chlorella *much more than mannitol solutions at −10 atmospheres. This occurred despite the purification of the polyethylene glycol by passage through exchange columns. The toxic properties, which developed some time after purification, increased with time of storage of solutions of polyethylene glycol 1540 at room temperature.*

Polyethylene glycols (PG) are now used extensively as osmotic agents to expose plants to a water deficit (among others *1*). Heavy metals may be present in the commercial polyethylene glycol ("Carbowax"), and most workers purify the PG either by dialysis or by passage over ion-exchange resins.

We first passed polyethylene glycol 1540 through the exchange resins Amberlite IR45 and Zeo Karb 225. The purified PG contained less than 0.3 parts of aluminum and 0.002 parts of chromium and copper (determined after ashing the carbowax and expressed as a percentage of the dry weight) per million. Water potentials were determined by psychrometer measurements (*2*).

Chlorella pyrenoidosa was treated with aqueous solutions of this purified PG1540, and effects on glucose- and acetate-induced respirations were compared with those of mannitol solutions at the same water potentials. Three days after purification, PG gave the same inhibition as mannitol, but after 3 weeks of storage PG1540 depressed the glucose-induced respiration more than mannitol did, and these specific effects increased with time of storage of the PG stock solution at room temperature (Table 1). In previous experiments (*3*) only freshly purified PG1540 was used for comparison with mannitol. After 6 months of storage of the PG, solutions of −10 atm gave "glucose respiration" at 3 percent of the control rates, this being considerably below the usual rate of endogenous respiration. Endogenous respiration is usually about 15 to 20 percent of "glucose respiration" and is not inhibited by mannitol solutions of −10 atm (*3*).

Table 1. Effects of time of storage at room temperature on the toxicity of "purified" PG1540 solutions. Glucose-induced respiration of *Chlorella* is expressed as a percentage of rates in control and mannitol solutions (−0.4 and −10 atm, respectively). A period of 70 to 200 minutes after application of the glucose and osmotic solution is used, since at 70 minutes control rates are nearly optimum and all experiments lasted for at least 200 minutes. Mannitol rates were assessed from other experiments done during the same series of tests. Temperature 25°C. Similar results were obtained for two different samples of PG1540, purchased in the United Kingdom and in Australia, respectively.

"Glucose respiration"	Time of storage				
	3 days	3 weeks	3 months	6 months	14 months
Control	70	40	18	3.5	4
Mannitol	100	56	26	5	7

The toxic effect was not confined to "glucose respiration." In *Chlorella*, both acetate-induced respiration and photosynthesis were inhibited much more by PG1540 stored for some months, than by PG1540 which was freshly purified by passage through an exchange column. Similar results were obtained for endogenous respiration of potato disks (Table 2). This latter experiment also showed that storage at 1°C prevented the formation of the inhibitor.

Psychrometer readings showed that the water potentials of PG1540 stock solution do not change after their original preparation. However, psychrometer readings would not be sensitive enough to detect the breakdown of some of the PG1540 molecules, which might have caused the toxic effect on *Chlorella*.

Fourteen months after purification of the PG1540, a sample of this purified solution was again passed through exchange resins. Its effect at −10 atm was compared with that of mannitol and of PG1540 purified only once, that is, 14 months earlier. The PG1540 that had been stored for 14 months severely inhibits "glucose respiration" of *Chlorella* (Fig. 1). Renewed purification completely removes the inhibitor. At the same time, PG1540 was purified only once and then used within 3 days of purification. The "glucose respiration" in the presence of this PG was the same as that induced by mannitol.

The chemical composition of the inhibitor is unknown, though it is presumably an ionized organic compound. The inhibitor might develop only in PG1540; for example, we found no

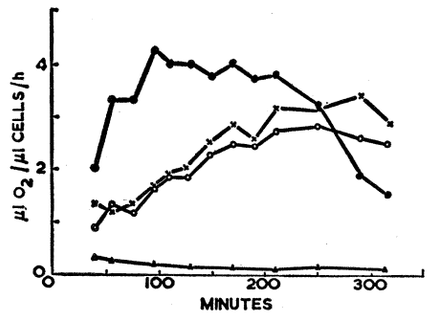

Fig. 1. Toxic effects of polyethylene glycol 1540 on glucose-induced respiration of *Chlorella pyrenoidosa*. (●———●) control, —0.4 atm; (×———×) mannitol, —10 atm; (○———○) PG1540, —10 atm purified both 14 months earlier and repurified prior to the test, by passage twice through exchange columns; (▲———▲) 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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26 September 1967; revised 5 December 1967

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.