TECHNICAL PAPERS

The Colchicine and Colchicine-like Reaction as a Possible Response to Enzymic Poisoning

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The gross effect of respiratory poisons and their antagonism by cellular extracts have been described for the root tips of the onion *Allium Cepa* L. (*6*). The present report is an extension of these investigations to the cytologic response of the roots to the same poisons and a comparison of these characteristics with those following exposure to colchicine.

Basic phenylmercuric nitrate and phenylmercuric hydroxide are strong polyploidogenic agents in the meristematic cells of the onion root, as was shown by the partial inhibition of spindle formation in dividing cells at the end of 5 hrs of exposure to 0.1 ppm of the mercurial compounds in water. Within 24 hrs after exposure a typical colchicine-like reaction developed cytologically, with the presence of blocked metaphases characterized by overcondensed chromosomes and diplochromosomes in acetocarmine staining of squash preparations. The same picture developed after 1 hr of exposure to 10 parts of poison in 1 million parts of water. Recovery from these sublethal effects of the poisons followed the same sequence as that observed following colchicine treatment—that is, lobed restitution nuclei, multinucleate cells, multipolar spindles, and normal mitosis in diploid and polyploid nuclei in the same tissue. So sensitive was the onion root meristem to the phenylmercuric nitrate and phenylmercuric hydroxide that exposure at $25 \pm 2°$ C to 1 part of the mercurial compound in 20, 40, and 80 million parts of water for 24 hrs resulted in partial inhibition of spindle formation and some tetraploid nuclei.

The cells first succumbing to the near-lethal dilutions of the phenylmercuric nitrate and phenylmercuric hydroxide were those just behind the meristem. In those roots recovering from continuous exposure to the mercurials at the lethal threshold (0.1 ppm of water), these cells enlarged isodiametrically instead of in the vertical plane, resulting in the so-called c-tumor or colchicine tumor of Levan (*5*). When these swollen tips were returned to water after 48–72 hrs in the poison, some continued to grow slowly in the clubbed form. In these tips the meristem was much reduced, and differentiation of the protoxylem strands extended to the promeristem. The same type of premature differentiation of protoxylem in onion roots after colchicine treatment was described by Eigsti (*3*) and was an irreversible cytoplasmic effect of the poisoning.

Bulbs bearing both untreated normal roots and roots with thickened (1–2 cm), elongated tips having polyploid nuclei as the result of previous exposure to sublethal concentrations of a mercurial were placed for 3 days over a solution of phenlymercuric nitrate (1 part in 3 million, 5.3×10^{-7}M). The normal roots became flaccid and died. The roots with hypertrophied tips remained turgid and grossly unchanged during the second period of exposure to the poison.

Ethyl mercury phosphate (*7, 8*) and ethyl mercuric chloride (*11*) have effects similar to those of phenylmercuric nitrate, phenylmercuric hydroxide, and colchicine on the meristem. The cells of the onion root were far more sensitive to phenylmercuric nitrate than were some microorganisms (*10*) and some enzyme systems (*1*). The action of the poison on the roots may be cumulative, as deduced by Dustin (*2*) from the work of Sass (*7*).

The observations reported herein would suggest that the colchicine and the colchicine-like reactions to mercurial poisons may be an adaptive mechanism of plant cells in the presence of an enzymic poison, as recently suggested for animal cells by Dustin (*2*), since basic phenylmercuric nitrate is a respiratory poison which probably attacks the –SH groups of succinic dehydrogenase and also inhibits lactic and glucose dehydrogenases as well as cytochrome oxidase and catalase (*1*).

An increase in the number of chromosomes in plants results in slower growth and lower growth energy (*5*). With the induction of polyploidy there is a decrease in the ratio of surface area to volume in protoplast and nucleus, whereas the reverse is true of the chromosomes. Levan (*4*) tested the sensitivity of diploid and tetraploid seedlings of barley, oats, and rye to colchicine, observing in a few instances that some concentrations affected the diploid cells earlier and more markedly than the tetraploid cells; he attributed the difference in response to the difference in the growth rate of the two types of cells. In our experiments the increased resistance of the hypertrophied root tips to re-exposure to the poisons may have been due to their reduced growth rate, premature differentiation, and the cuboidal form of the cells behind the meristem.

The presence of polyploidy and of polytene chromosomes in some tissues of diploid plants and animals would suggest an adaptation of the cell to an unfavorable environment for enzyme reactions. The cancer process is considered by Spencer (*9*) as a type of cell adaptation to an unfavorable environment, a survival mechanism, and Levan and Ostergren (*5*) have attempted to equate the actions of colchicine and carcinogens. The similarity of the colchicine reaction and the colchicine-like reaction to

mercurial respiratory poisons in onion root tips emphasizes the difference between the colchicine response and cytologic characteristics in carcinogenesis, since colchicine produces (a) an immediate reversible inhibitory effect on the cytoplasm of dividing cells without arresting chromosomal reproduction, (b) a delayed irreversible decrease in the rate of cell division, and (c) an increase in the rate of cellular differentiation.

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The Crystalline Form of Sodium Ascorbate[1]

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The desirability of a stable, neutral, dry salt of ascorbic acid had been felt virtually from the moment of the discovery that ascorbic acid was vitamin C, but the rapid decomposition of ascorbic acid in the presence of alkali seemed to make this a hopeless search. Sherman (*3*), Thorpe (*4*), and others described the destructive action of alkali on ascorbic acid. Karrer (*1*) in 1933 had tried to secure a dry salt by reacting sodium ethylate in ethyl alcohol but indicated that rapid decomposition readily resulted. So certain did it appear that a stable sodium salt would not exist that in the intervening years not a single further publication appeared on the subject, nor was any dry sodium ascorbate made available.

A therapeutically useful crystalline sodium ascorbic was finally attained through reaction of sodium methylate in methyl alcohol (*2*). Surprisingly, this yielded a product of remarkable stability exceeding that of ascorbic acid itself. After 500 hrs aging at 45° C in closed glass containers, the sodium ascorbate showed no decline in ascorbic acid potency. Pure crystals were obtained which could successfully be used for seeding concentrated syrups of sodium ascorbate with a high yield of crystalline sodium ascorbate. The preparation of sodium ascorbate with sodium methylate in methyl alcohol was conducted as follows.

[1] This research was conducted under a grant from the Physiological Chemicals Company, Inc., who also supplied the sodium ascorbate (Natri-C).

Eighty-eight gm (0.5 mole) of ascorbic acid was dissolved in 600 cc of hot absolute methyl alcohol. While still hot, it was treated under stirring with 250 cc of a warm solution of sodium methylate containing 12.5 gm of sodium (theory, 11.5 gm). The combined solutions were stirred until the resulting precipitate of sodium ascorbate turned crystalline. This took about 15 min. The sodium ascorbate was then filtered with suction and washed with a little methyl alcohol. It could be dried *in vacuo* at a temperature as high as 100° C. The yield was 95% and the product 100% pure.

Once stable crystals were obtained in the laboratory, seeding was undertaken to perfect the formation and was readily accomplished despite the alkaline nature of the reaction. It seems to be a common laboratory experience that, once the crystals are obtained in the laboratory, solutions previously difficult to crystallize do so very readily.

The explanation for the formation of stable crystals may be sought in the location of the neutralization. The *U.S.P.* (XIII, p. 898) gives the structural formula of sodium ascorbate as $CH_2OH\ (CHOH)_2\ COH : COHCOONa$, mol.wt. 216.13. It is commonly believed, however, that the neutralization involves a hydroxyl group and that there would be quite a difference in reactivity, depending upon whether the second or third hydroxyl is the location of the neutralization. The third carbon is apparently much more reactive than the second, and its neutralization may produce greater stability. Karrer (*1*) points out that an excess of ethylate solution must be avoided, as otherwise the yield is greatly reduced. He states that "apparently there then occurs also neutralization of the enolic hydroxyl and decomposition processes of the sensitive substance also occur." Karrer's interpretation would include not only neutralization of the carboxyl but also, in some instances, of the enolic hydroxyl, thus suggesting that two reactions may proceed simultaneously. With the methylate, apparently a single hydroxyl reaction occurs with a uniform hydroxyl reaction of remarkable stability. This would also seem to be the case from the petrographic studies of the crystals, which showed cryptocrystalline formation with the ethylate, a good degree of pure crystals with the methylate and on seeding from the methylate from concentrated syrup.

The crystals showed the following analysis:

Rotation, + 102.99.

Iodine titration, ascorbic acid, 87.55; theory, 88.9

Analysis:

	Found	*Theory*
Sodium	11.33	11.61
Carbon	35.93	36.37
Hydrogen	3.68	3.58

Analysis indicates about 1% water held by crystals.

Further procedures were developed using sodium hydroxide, sodium carbonate, or sodium hydride with similar results.

Petrographic studies conducted by Dr. Wilbur G. Valentine showed the variety of crystallization that may occur and throws light on the possible explanation that the stability of sodium ascorbate may depend also on the