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

state of aggregation. A similar instance is known to occur in the remarkable stability of the dihydrate of calcium ascorbate as compared to the nonhydrated form, as shown in a previous paper.

X-ray diffraction studies by Dr. Fankuchen gave the following values:

| *d in Angstrom Units* | *Intensities* |
|---|---|
| 9.93 | M |
| 4.82 | S |
| 4.52 | V.W. |
| 4.27 | V.W. |
| 4.05 | V.S. |
| 3.66 | S |
| 3.38 | S |
| 3.20 | W |
| 3.11 | S |
| 2.94 | W |
| 2.80 | M |
| 2.61 | M |
| 2.40 | W |
| 2.30 | W |
| 2.26 | M |
| 2.14 | M |
| 2.08 | M |
| 1.98 | M |
| 1.91 | W |
| 1.81 | W |

X-ray diffraction studies were made comparing the material made by the ethyl alcohol procedure. Methyl alcohol and water-solution crystals seeded with the methyl alcohol crystals. The material made from the

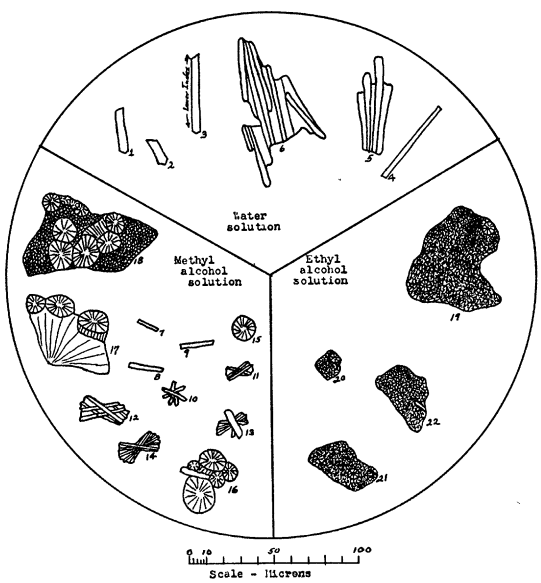


FIG. 1

ethyl alcohol, while having the same general structure as the others, showed a difference on some lines due, obviously, to an impurity. This was particularly true of the line 2.95 A, which is appreciably stronger in the water crystals than in the ethyl alcohol material. There is also a weak line in the water crystals at 6.16 A which is not observed in the other material.

Camera lucida drawings of representative fragments from each of the three samples studied are shown in Fig. 1.

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## Effect of Hyaluronidase and of Hyaluronic Acid on Cultures of Trypanosomes, *Leishmania,* and *Amoebae*


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Hyaluronic acid, a complex polysaccharide, and its specific enzyme, hyaluronidase, form a biological system of mucoid structure common to bacterial and animal (including human) species (*1, 2*). In the present work we have sought to determine the respective effects of the acid and the enzyme *in vitro* upon *Leishmania donovani, Trypanosoma cruzi,* and *Endamoeba histolytica,* using hyaluronidase from bovine testes and hyaluronic acid from human umbilical cord.[1]

*L. donovani* and *T. cruzi* were grown on Seneca's medium (*3*) for 7–10 days. Direct observations were then made upon organisms suspended in saline containing 50 units of hyaluronidase/cc or suspended in 0.5% hyaluronic acid solution for varying lengths of time from 15 min to 4 days. Observations were made on cultures in 5 cc of physiological saline containing 1½, 2½, 5, and 10 units of hyaluronidase, inoculated from the 7- to 10-day cultures and serially transplanted weekly, to identical media and concentrations, for 12 transplants, and on similar 5-cc cultures containing 5 or 1 mg of hyaluronic acid in 5 cc, transplanted weekly for 5 transplants. Microscopic study was done to find the number, viability, and motility of the respective organisms as various times.

*E. histolytica* of the Denton strain was taken from culture, suspended for direct observation in saline containing 5 units of hyaluronidase/cc, and observed for 48 hrs. Observations were made on cultures prepared by inoculating *Amoebae* with 5 cc of buffered saline containing 1½, 2½, 5, and 10 units of hyaluronidase, overlaid on *Amoeba* culture medium; subcultures were done 3 times weekly, covering 50 generations over a period of 4 months. Similar direct observations were made using 0.5, 0.25, 0.125, 0.0625, and 0.03125% hyaluronic acid in saline and in cultures treated with 1 mg of hyaluronic acid in 5 cc of saline, with subcultures to 5 generations.

The results were as follows:

*Hyaluronidase. L. donovani* and *T. cruzi* were unaffected by direct treatment with hyaluronidase or by

[1] Preparations were made by the Chemical Division of the Schering Corporation.