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

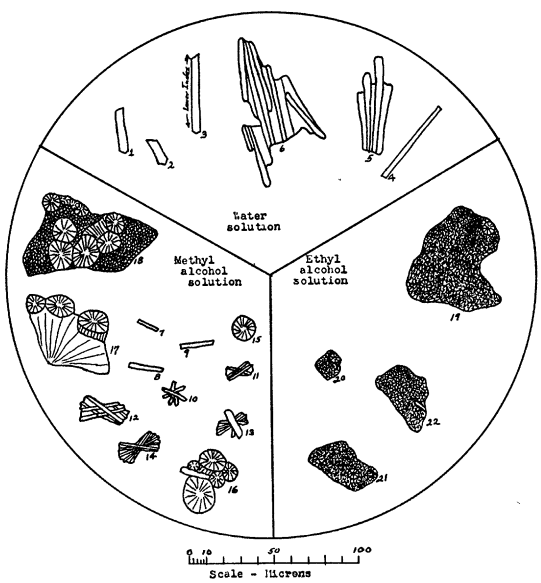

FIG. 1

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.

culturing in its presence; rate of multiplication, vitality, and motility remained the same as in control preparations. *E. histolytica* suspended in solutions of hyaluronidase showed a type of hypermotility, but no changes in vitality or structure. Serial transplantations over 50 generations of *Amoebae* showed that there was enhancement of growth in the presence of hyaluronidase, so that more abundant cultures resulted.

Hyaluronic acid. Hemoflagellates suspended in 0.5% hyaluronic acid showed a steady reduction in motility, particularly of *Leishmania*, but without evidence that the organisms were killed. Serial cultures in the presence of hyaluronic acid showed no effect on the rate of multiplication, vitality, or motility of the organisms over 5 generations. *E. histolytica* exposed directly to hyaluronic acid showed certain structural changes, consisting of hyalinization of the organism and progressive degeneration and rupture, until all the *Amoebae* disappeared. These changes were complete in 15 min with the 0.5% solution of hyaluronic acid and in 70 min with the 0.25% solution; they consisted only of progressive weakening of ameboid activity in 2 hrs with 0.125% solution, while weaker solutions had no effect in 3 hrs. *Amoebae* grown in the presence of 5 mg of hyaluronic acid/5 cc were killed in the 3rd generation; 1 mg/5 cc, in the 5th generation.

Hyaluronidase and hyaluronic acid have indifferent effect upon the hemoflagellates tested. These substances are, however, markedly stimulating and markedly toxic, respectively, to growing cultures of *E. histolytica*; both findings suggest a certain usefulness in culturing *Amoebae* or in attempting to treat amebic infections. While it is not known that *Amoebae* utilize hyaluronidase to invade the host intestine, the observed effect of hyaluronic acid suggests that, if it be used therapeutically, an action both upon the protozoon and upon its power to invade the tissues might be obtained, the latter by inhibition of hyaluronidase possibly secreted by the invader.

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Responses of Cuttings, Seeds, and Flowers to Dithiobiuret

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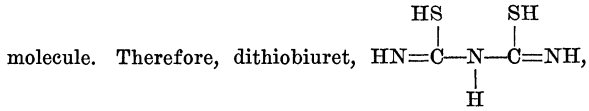
Molecular structures that embody reduced forms of sulfur (such as –SH groups) and of nitrogen are of general interest to physiologists, and it is well known that the activity of sulfhydryl groups is influenced by the number and position of neighboring nitrogen atoms in the molecule. Therefore, dithiobiuret,  , since it embodies two –SH groups symmetically arranged with respect to three –NH groups, and since it is capable of serving not only as an H donor but as a source of S and of N as well, should be expected to possess some interesting biological properties. The compound is easily oxidized with loss of two hydrogens to form a 5-membered  ring system (*3*). The reaction proceeds rapidly and reversibly over the pH range 0.05–5.2 (*3*). Dithiobiuret operates as a reducing agent at hydrogen-ion concentrations within the biological range also, as is testified by its ability even in low concentrations (0.0005 M) to decolorize rapidly toluylene blue in solutions ranging up to pH values of 7.5 (higher pH values not studied). This dye is but slowly reduced by cysteine (*1*).

This report presents some observations on the response of vine cuttings, germinating seeds, and cut flowers to dithiobiuret,[1] which for convenience will be designated as DTB in the remainder of this report.

Vine cuttings. Cuttings of grape vines (*Vitis treleasi*) with their bases immersed in distilled water normally developed, within 2 weeks, shoots from the upper bud and roots from the lower node. Cuttings similarly placed in solutions containing 20–80 mg of DTB/liter of distilled water developed short roots and stunted shoots from buds, irrespective of the relative position of the bud on the stem. When such cuttings were transferred to distilled water, the roots promptly elongated. Similar tendencies have been observed with cuttings of *Ficus gnaptholocarpa* (*2*).

Cuttings in 0.05 M solution of KH_2PO_4 did not develop any roots in 2 weeks; DTB at 20–80 mg/liter in the phosphate buffer did not invert polarity but had a striking effect in promoting the differentiation of root initials not only from the basal node but all along the basal internodes. Potassium acid phosphate retarded early development of roots and of buds as compared with distilled water, but favored cambial growth at the base of the cutting as well as differentiation of floral organs. Both of the latter effects were enhanced by the presence of DTB. In KH_2PO_4 the increase in diameter was accompanied by longitudinal splitting of the bark and later on by differentiation of root initials, which attained a length of about 10 cm after 3 weeks. Conversely, in KH_2PO_4 + DTB (20 mg/liter) a few root initials differentiated into roots, and these elongated, but slowly, averaging 2.5 cm after 3 weeks. Most of the root initials coalesced into an undifferentiated mass of cells, while the base of each cutting swelled into a tumor-like mass.

Early growth of seedlings under semianaerobic conditions. Seeds of rice (*Oryza sativa* var. Calora) were immersed under 3 cm of a simple nutrient solution (KH_2PO_4, 0.03 M; $Ca(NO_3)_2$, 0.02 M; and $MgSO_4 \cdot 7H_2O$, 0.01 M) or of nutrient plus DTB. Addition of DTB in concentrations less than 10 mg/liter had a depressing effect on germination and on growth of roots and coleoptiles. Concentrations of from 10 to 25 mg/liter were strikingly beneficial, leaves developing to a length of 70 mm in 17

[1] We wish to thank the American Cyanamid Company, New York City, for samples of dithiobiuret; L. Flint, Louisiana State University, for seeds of rye grass; and H. P. Olmo, University of California College of Agriculture, for the vine cuttings.